(19)日本国特許庁（ＪＰ）　(12) 公開特許公報（Ａ）　(11)特許出願公開番号

特開平11－187900

(43)公開日　平成11年(1999) 7月13日

(51)Int.Cl.^{6}	識別記号	ＦＩ	
Ｃ１２Ｑ　1/68		Ｃ１２Ｑ　1/68	Ａ
Ｇ０１Ｎ　33/50		Ｇ０１Ｎ　33/50	Ｐ
33/543	５２５	33/543	５２５Ｇ
33/566		33/566	
// Ｃ１２Ｎ　15/09		Ｃ１２Ｎ　15/00	Ａ

審査請求　未請求　請求項の数219　ＯＬ　（全 29 頁）

(21)出願番号　特願平10－209923

(22)出願日　平成10年(1998) 7月24日

(31)優先権主張番号　特願平9－207837

(32)優先日　平9(1997) 8月1日

(33)優先権主張国　日本（ＪＰ）

(31)優先権主張番号　特願平9－287046

(32)優先日　平9(1997)10月20日

(33)優先権主張国　日本（ＪＰ）

(71)出願人　000001007
キヤノン株式会社
東京都大田区下丸子３丁目30番２号

(72)発明者　山本　伸子
東京都大田区下丸子３丁目30番２号　キヤノン株式会社内

(72)発明者　岡本　尚志
東京都大田区下丸子３丁目30番２号　キヤノン株式会社内

(72)発明者　鈴木　智博
東京都大田区下丸子３丁目30番２号　キヤノン株式会社内

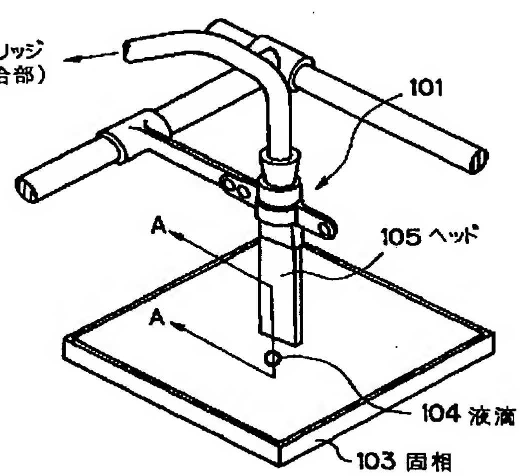
(74)代理人　弁理士　若林　忠　（外４名）

(54)【発明の名称】プローブの固相へのスポッティング方法、プローブアレイとその製造方法、及びそれを用いた標的物質の検出方法、標的物質の構造の特定化方法

(57)【要約】

【課題】　高密度で効率良くプローブを固相表面にスポッティングする方法を提供する。

【解決手段】　プローブを含む液体をインクジェット法により液滴として固相に付着させて、プローブを含むスポットを固相上に形成する。



【特許請求の範囲】

【請求項１】　標的物質に対して特異的に結合可能であるプローブを含む液体をインクジェット法により固相表面に供給し、該固相表面に付着させる工程を有することを特徴とするプローブの固相へのスポッティング方法。

【請求項２】　該プローブが一本鎖核酸プローブである請求項１記載のスポッティング方法。

【請求項３】　該一本鎖核酸プローブが一本鎖ＤＮＡプローブを含む請求項２記載のスポッティング方法。

【請求項４】　該一本鎖核酸プローブがＲＮＡプローブを含む請求項２記載のスポッティング方法。

【請求項５】　該一本鎖核酸プローブが一本鎖ＰＮＡプローブを含む請求項２記載のスポッティング方法。

【請求項６】　該固相表面と該一本鎖核酸プローブとが各々官能基を有し、該官能基同士は接触によって反応するものである請求項２記載のスポッティング方法。

【請求項７】　該固相表面が有する官能基がマレイミド基であって、該一本鎖核酸プローブの有する官能基がチオール（ＳＨ）基である請求項６記載のスポッティング方法。

【請求項８】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とＮ－（６－マレイミドカプロイロキシ）スクシイミドとを反応させて導入したものである請求項７記載のスポッティング方法。

【請求項９】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とスクシイミジル－４－（マレイミドフェニル）ブチレートとを反応させて導入したものである請求項７記載のスポッティング方法。

【請求項１０】　該ガラス基板上のマレイミド基と該一本鎖核酸のチオール基とを少なくとも３０分反応させる請求項７記載のスポッティング方法。

【請求項１１】　該一本鎖核酸が末端にチオール基を有する一本鎖ＰＮＡプローブを含み、該マレイミド基と該チオール基とを少なくとも２時間以上反応させる請求項１０記載のスポッティング方法。

【請求項１２】　該一本鎖ＰＮＡプローブ末端のチオール基が、一本鎖ＰＮＡプローブのＮ末端側へのシステインの結合によって導入されているものである請求項１１記載のスポッティング方法。

【請求項１３】　該固相表面が有する官能基がエポキシ基であって、該一本鎖核酸プローブの有する官能基がアミノ基である請求項６記載のスポッティング方法。

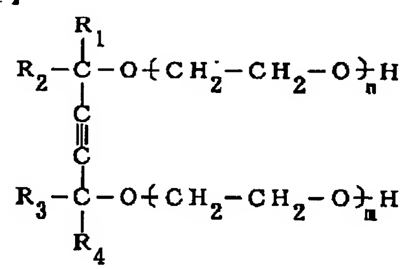
【請求項１４】　該固相がガラス板であり、また該エポキシ基は、該ガラス板の表面にエポキシ基を分子内に有するシラン化合物を塗布し、該化合物と該ガラス板とを反応させて導入したものである請求項１３記載のスポッティング方法。

【請求項１５】　該エポキシ基は、エポキシ基を有するポリグリシジルメタクリレートの該固相上への塗布によって導入したものである請求項１３記載のスポッティング方法。

【請求項１６】　該液体が、該液体に対して尿素を５～１０ｗｔ％、グリセリンを５～１０ｗｔ％、チオジグリコールを５～１０ｗｔ％、及びアセチレンアルコールを１ｗｔ％含んでいる請求項１記載のスポッティング方法。

【請求項１７】　該アセチレンアルコールが下記一般式（Ｉ）で示される構造を有するものである請求項１６記載のスポッティング方法。

【化１】



（Ｉ）

（上記式中、R_1、R_2、R_3及びR_4はアルキル基を表わし、ｍおよびｎは夫々整数を表わし、ｍ＝０かつｎ＝０、もしくは１≦ｍ＋ｎ≦３０であって、ｍ＋ｎ＝１の場合はｍまたはｎは０である。）

【請求項１８】　該液体中の該一本鎖核酸プローブの濃度が０．０５～５００μＭである請求項２記載のスポッティング方法。

【請求項１９】　該液体中の該一本鎖核酸プローブの濃度が２～５０μＭである請求項１８記載のスポッティング方法。

【請求項２０】　該一本鎖核酸プローブの長さが２～５０００塩基長である請求項２記載のスポッティング方法。

【請求項２１】　該一本鎖核酸プローブの長さが２～６０塩基長である請求項２０記載のスポッティング方法。

【請求項２２】　該インクジェット法がバブルジェット法である請求項１記載のスポッティング方法。

【請求項２３】　該プローブが特定のアミノ酸配列を有するオリゴペプチド或いはポリペプチドである請求項１記載のスポッティング方法。

【請求項２４】　該プローブが蛋白質である請求項１記載のスポッティング方法。

【請求項２５】　該蛋白質が抗体である請求項２４記載のスポッティング方法。

【請求項２６】　該蛋白質が酵素である請求項２４記載のスポッティング方法。

【請求項２７】　該プローブが酵素である請求項１記載のスポッティング方法。

【請求項２８】　該液体を、該固相上に１平方インチあたり１００００個以上の密度で、互いに独立したスポッ

トとなるようにスポッティングする請求項１記載のスポッティング方法。
【請求項２９】　該固相は表面が平坦であって、且つ均一な表面特性を有している請求項１記載のスポッティング方法。
【請求項３０】　隣接するスポットの間隔が該スポットの最大幅以上となるようにスポッティングする請求項２９記載のスポッティング方法。
【請求項３１】　該固相表面の、スポット以外の部位に核酸が付着しないようにブロッキングされている請求項３０記載のスポッティング方法。
【請求項３２】　該ブロッキングが牛血清アルブミンによって達成されている請求項３１記載のスポッティング方法。
【請求項３３】　該固相が表面にパターン状に配置されたマトリクスで区画され、パターン状に露出してなる該固相表面を底面とする複数のウェルを備え、該液体を各々のウェルに供給する請求項１記載のスポッティング方法。
【請求項３４】　該固相が光学的に透明であり、該マトリクスが遮光性である請求項３３記載のスポッティング方法。
【請求項３５】　該マトリクスが樹脂を含む請求項３３記載のスポッティング方法。
【請求項３６】　該マトリクスの表面が疎水性である請求項３３記載のスポッティング方法。
【請求項３７】　該ウェルの底面が親水性である請求項３３記載のスポッティング方法。
【請求項３８】　該マトリクスの厚さが１～２０μｍである請求項３３記載のスポッティング方法。
【請求項３９】　該ウェルの最長幅が２００μｍである請求項３３記載のスポッティング方法。
【請求項４０】　該マトリクスの幅が、該ウェルの最長幅の１／２～２倍である請求項３３記載のスポッティング方法。
【請求項４１】　固相表面の複数の部位に互いに独立しているプローブのスポットを１平方インチ内に１０００個以上の密度で備えていることを特徴とするプローブアレイ。
【請求項４２】　該固相は平坦な表面を有し、かつ均一な表面特性を有している請求項４１記載のプローブアレイ。
【請求項４３】　該プローブが一本鎖核酸プローブである請求項４２記載のプローブアレイ。
【請求項４４】　該一本鎖核酸プローブが一本鎖ＤＮＡプローブを含む請求項４３記載のプローブアレイ。
【請求項４５】　該一本鎖核酸プローブが一本鎖ＲＮＡプローブを含む請求項４３記載のプローブアレイ。
【請求項４６】　該一本鎖核酸プローブが一本鎖ＰＮＡプローブを含む請求項４３記載のプローブアレイ。
【請求項４７】　該固相表面と一本鎖核酸プローブとが各々有する官能基同士の反応によって該一本鎖核酸プローブが該固相表面に共有結合によって結合している請求項４３記載のプローブアレイ。
【請求項４８】　該固相表面が有する官能基がマレイミド基であって、該一本鎖核酸プローブの有する官能基がチオール（ＳＨ）基である請求項４７記載のプローブアレイ。
【請求項４９】　該一本鎖核酸プローブが一本鎖ＰＮＡプローブであり、Ｎ末端側にシステイン残基を有する請求項４８記載のプローブアレイ。
【請求項５０】　該固相表面が有する官能基がエポキシ基であり、該一本鎖プローブの有する官能基がアミノ基である請求項４７記載のプローブアレイ。
【請求項５１】　該スポットが、核酸プローブを含む液体の該固相上への付与によって形成されたものである請求項４２記載のプローブアレイ。
【請求項５２】　該プローブが特定のアミノ酸配列を有するオリゴペプチド或いはポリペプチドである請求項４２記載のプローブアレイ。
【請求項５３】　該プローブが蛋白質である請求項４２記載のプローブアレイ。
【請求項５４】　該蛋白質が抗体である請求項５３記載のプローブアレイ。
【請求項５５】　該蛋白質が酵素である請求項５３記載のプローブアレイ。
【請求項５６】　該プローブが抗原である請求項４２記載のプローブアレイ。
【請求項５７】　該スポットの間隔が該スポットの最長幅以上である請求項４２記載のプローブアレイ。
【請求項５８】　該固相が表面にパターン状に配置されたマトリクスで区画され、パターン状に露出してなる該固相表面を底面とする複数のウェルを備え、各々のスポットが各々のウェルの位置と一致している請求項４１記載のプローブアレイ。
【請求項５９】　該プローブが一本鎖核酸プローブである請求項５８記載のプローブアレイ。
【請求項６０】　該一本鎖核酸プローブが一本鎖ＤＮＡプローブを含む請求項５９記載のプローブアレイ。
【請求項６１】　該一本鎖核酸プローブがＲＮＡプローブを含む請求項５９記載のプローブアレイ。
【請求項６２】　該一本鎖核酸プローブが一本鎖ＰＮＡプローブを含む請求項５９記載のプローブアレイ。
【請求項６３】　該固相表面と一本鎖核酸プローブとが各々有する官能基同士の反応によって該一本鎖核酸プローブが該固相表面に共有結合によって結合している請求項６２記載のプローブアレイ。
【請求項６４】　該固相表面が有する官能基がマレイミド基であって、該一本鎖核酸プローブの有する官能基がチオール（ＳＨ）基である請求項６３記載のプローブア

レイ。

【請求項６５】　該一本鎖核酸プローブが一本鎖ＰＮＡプローブであり、Ｎ末端側にシステイン残基を有する請求項６４記載のプローブアレイ。

【請求項６６】　該固相表面が有する官能基がエポキシ基であり、該一本鎖プローブの有する官能基がアミノ基である請求項６３記載のプローブアレイ。

【請求項６７】　該スポットが、該核酸プローブを含む液体の該固相上への付与によって形成されたものである請求項５８記載のプローブアレイ。

【請求項６８】　該プローブが特定のアミノ酸配列を有するオリゴペプチド或いはポリペプチドである請求項５８記載のプローブアレイ。

【請求項６９】　該プローブが蛋白質である請求項５８記載のプローブアレイ。

【請求項７０】　該蛋白質が抗体である請求項６９記載のプローブアレイ。

【請求項７１】　該蛋白質が酵素である請求項６９記載のプローブアレイ。

【請求項７２】　該プローブが抗原である請求項５８記載のプローブアレイ。

【請求項７３】　該マトリクスが遮光性である請求項５８記載のプローブアレイ。

【請求項７４】　該固相が光学的に透明である請求項７３記載のプローブアレイ。

【請求項７５】　該マトリクスが樹脂を含む請求項５８記載のプローブアレイ。

【請求項７６】　該ウェルにのみ該プローブが付着している請求項５８記載のプローブアレイ。

【請求項７７】　該マトリクスの厚さが１～２０μｍである請求項５８記載のプローブアレイ。

【請求項７８】　該ウェルの最長幅が２００μｍである請求項５８記載のプローブアレイ。

【請求項７９】　該ウェルの間隔が、該ウェルの最長幅の１／２～２倍である請求項５８記載のプローブアレイ。

【請求項８０】　互いに異なる種類のプローブからなるスポットを少なくとも２つ有する請求項４１記載のプローブアレイ。

【請求項８１】　固相表面の複数の箇所に独立して、標的物質に対して特異的に結合可能であるプローブを含むスポットを有するプローブアレイの製造方法であって、該プローブを含む液体をインクジェット法を用いて該固相表面の所定の位置に供給し、付着させる工程を有することを特徴とするプローブアレイの製造方法。

【請求項８２】　該プローブが一本鎖核酸プローブである請求項８１記載の製造方法。

【請求項８３】　該一本鎖核酸プローブが一本鎖ＤＮＡプローブを含む請求項８２記載の製造方法。

【請求項８４】　該一本鎖核酸プローブがＲＮＡプローブを含む請求項８２記載の製造方法。

【請求項８５】　該一本鎖核酸プローブが一本鎖ＰＮＡプローブを含む請求項８２記載の製造方法。

【請求項８６】　該固相表面と該一本鎖核酸プローブとが各々官能基を有し、該官能基同士は接触によって反応するものである請求項８２記載の製造方法。

【請求項８７】　該固相表面が有する官能基がマレイミド基であって、該一本鎖核酸プローブの有する官能基がチオール（ＳＨ）基である請求項８６記載の製造方法。

【請求項８８】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とＮ－（６－マレイミドカプロイロキシ）スクシイミドとを反応させて導入したものである請求項８７記載の製造方法。

【請求項８９】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とスクシイミジルー４－（マレイミドフェニル）ブチレートとを反応させて導入したものである請求項８７記載の製造方法。

【請求項９０】　該ガラス基板上のマレイミド基と該一本鎖核酸のチオール基とを少なくとも３０分反応させる請求項８７記載の製造方法。

【請求項９１】　該一本鎖核酸が末端にチオール基を有する一本鎖ＰＮＡプローブを含み、該マレイミド基と該チオール基とを少なくとも２時間以上反応させる請求項８７記載の製造方法。

【請求項９２】　該一本鎖ＰＮＡプローブ末端のチオール基が、一本鎖ＰＮＡプローブのＮ末端側へのシステインの結合によって導入されているものである請求項９１記載の製造方法。

【請求項９３】　該固相表面が有する官能基がエポキシ基であって、該一本鎖核酸プローブの有する官能基がアミノ基である請求項８６記載の製造方法。

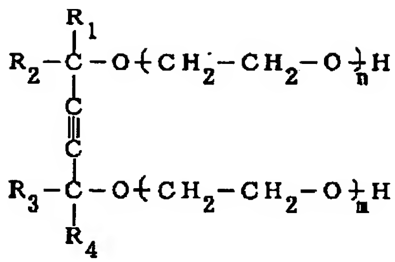
【請求項９４】　該固相がガラス板であり、また該エポキシ基は、該ガラス板の表面にエポキシ基を分子内に有するシラン化合物を塗布し、該化合物と該ガラス板とを反応させて導入したものである請求項９３記載の製造方法。

【請求項９５】　該エポキシ基は、エポキシ基を有するポリグリシジルメタクリレート樹脂の該固相上への塗布によって導入したものである請求項９３記載の製造方法。

【請求項９６】　該液体が、該液体に対して尿素を５～１０ｗｔ％、グリセリンを５～１０ｗｔ％、チオジグリコールを５～１０ｗｔ％、及びアセチレンアルコールを１ｗｔ％含んでいる請求項８２記載の製造方法。

【請求項９７】　該アセチレンアルコールが下記一般式（Ｉ）で示される構造を有するものである請求項９６記載の製造方法。

【化２】



（I）

（上記式中、R_1、R_2、R_3及びR_4はアルキル基を表わし、mおよびnは夫々整数を表わし、m＝０かつn＝０、もしくは１≦m＋n≦３０であって、m＋n＝１の場合はmまたはnは０である。）

【請求項９８】　該液体中の該一本鎖核酸プローブの濃度が０．０５～５００μMである請求項８２記載の製造方法。

【請求項９９】　該液体中の該一本鎖核酸プローブの濃度が２～５０μMである請求項９８記載の製造方法。

【請求項１００】　該一本鎖核酸プローブの長さが２～５０００塩基長である請求項８２記載の製造方法。

【請求項１０１】　該一本鎖核酸プローブの長さが２～６０塩基長である請求項１００記載の製造方法。

【請求項１０２】　該インクジェット法がバブルジェット法である請求項８１記載の製造方法。

【請求項１０３】　該一本鎖核酸プローブを含む液体を該固相上に、１平方インチあたり１００００個以上の密度で、互いに独立したスポットとなるようにスポッティングする請求項８１記載の製造方法。

【請求項１０４】　該プローブが特定のアミノ酸配列を有するオリゴペプチド或いはポリペプチドである請求項８１記載の製造方法。

【請求項１０５】　該プローブが蛋白質である請求項８１記載の製造方法。

【請求項１０６】　該蛋白質が抗体である請求項１０５記載の製造方法。

【請求項１０７】　該蛋白質が酵素である請求項１０５記載の製造方法。

【請求項１０８】　該プローブが抗原である請求項８１記載の製造方法。

【請求項１０９】　該固相は表面が平坦であって、且つ均一な表面特性を有している請求項８１記載の製造方法。

【請求項１１０】　固相に該一本鎖核酸を固定させた後、該一本鎖核酸が固定化されている部位以外の部位に核酸が付着しないようにブロッキングを行なう請求項１０９記載の製造方法。

【請求項１１１】　該ブロッキングが、該一本鎖核酸が固定された固相をウシ血清アルブミン水溶液に浸す工程を有する請求項１１０記載の製造方法。

【請求項１１２】　該ウシ血清アルブミンの濃度が０．１～５％である請求項１１１記載の製造方法。

【請求項１１３】　該固相のウシ血清アルブミン水溶液への浸漬を少なくとも２時間行なう請求項１１１記載の製造方法。

【請求項１１４】　隣接するスポットの間隔が該スポットの最大幅以上となるようにスポッティングする請求項１０９記載の製造方法。

【請求項１１５】　該固相が表面にパターン状に配置されたマトリクスで区画され、パターン状に露出してなる該固相表面を底面とする複数のウェルを備え、該液体を各々のウェルに供給する請求項８１記載の製造方法。

【請求項１１６】　該固相が光学的に透明であり、該マトリクスが遮光性である請求項１１５記載の製造方法。

【請求項１１７】　該マトリクスが樹脂を含む請求項１１５記載の製造方法。

【請求項１１８】　該マトリクスの表面が疎水性である請求項１１５記載の製造方法。

【請求項１１９】　該ウェルの底面が親水性である請求項１１５記載の製造方法。

【請求項１２０】　該ウェルの最長幅が２００μmである請求項１１５記載の製造方法。

【請求項１２１】　該マトリクスの幅が、該ウェルの最長幅の１／２～２倍である請求項１１５記載の製造方法。

【請求項１２２】　該マトリクスの厚さが１～２０μmである請求項１１５記載の製造方法。

【請求項１２３】　該マトリクスパターンを、フォトリソグラフィー法によって形成する請求項１１５記載の製造方法。

【請求項１２４】　該フォトリソグラフィー法が、該基板の第１の表面に樹脂層を形成し、該樹脂層上にフォトレジスト層を形成し、該フォトレジスト層を該マトリクスパターンに対応するようにパターン状に露光し、現像してフォトレジストのパターンを該樹脂層上に形成する工程；及び該フォトレジストのパターンをマスクとして該樹脂層をパターニングした後、該フォトレジストのパターンを除去する工程、を有する請求項１２３記載の製造方法。

【請求項１２５】　該フォトリソグラフィー法が、該基板の第１の表面に感光性樹脂層を形成し、該感光性樹脂層を該マトリクスパターンに対応するようにパターン状に露光し、現像する工程を有する請求項１２３記載の製造方法。

【請求項１２６】　該感光性樹脂層が、UVレジスト、DEEP－UVレジスト、または紫外線硬化樹脂を含むものである請求項１２５記載の製造方法。

【請求項１２７】　該UVレジストが、環化ポリイソプレン－芳香族ビスアジド系レジスト、フェノール樹脂－芳香族アジド化合物系レジスト、またはノボラック樹脂－ジアゾナフトキノン系レジストである請求項１２６記載の製造方法。

【請求項１２８】　該ＤＥＥＰ－ＵＶレジストが放射線分解型レジストまたは溶解抑制剤系レジストである請求項１２６記載の製造方法。

【請求項１２９】　該放射線分解型ポリマーレジストが、ポリメチルメタクリレート、ポリメチレンスルホン、ポリヘキサフルオロブチルメタクリレート、ポリメチルイソプロペニルケトンまたは臭化ポリー１ートリメチルシリルプロピンから選ばれる少なくとも１つである請求項１２８記載の製造方法。

【請求項１３０】　該溶解抑制剤系レジストが、コール酸ｏ－ニトロベンジルエステルである請求項１２８記載の製造方法。

【請求項１３１】　該ＤＥＥＰ－ＵＶレジストがポリビニルフェノールー３，３’ージアジドジフェニルスルホンまたはメタクリル酸グリシジルである請求項１２６記載の製造方法。

【請求項１３２】　該感光性樹脂層のパターニングによって形成したマトリクスパターンを、更にポストベークして該マトリクスパターンの撥水性を向上させる請求項１２５記載の製造方法。

【請求項１３３】　該プローブが有する官能基と共有結合を形成可能な官能基を該固相表面に、該ウェルの形成に先立って導入する請求項１１５記載の製造方法。

【請求項１３４】　該プローブが有する官能基と共有結合を形成可能な官能基を該固相表面に、該ウェルの形成後に導入する請求項１１５記載の製造方法。

【請求項１３５】　該固相表面に、該官能基を導入するための化合物を含む溶液を該ウェルに付与する請求項１３４記載の製造方法。

【請求項１３６】　該溶液のウェルへの付与をインクジェット法を利用して行なう請求項１３５記載の製造方法。

【請求項１３７】　該溶液がエポキシ基又はアミノ基を分子内に有するシラン化合物を含むシランカップリング剤である請求項１３６記載の製造方法。

【請求項１３８】　該溶液がガラス基板上のアミノ基と反応してガラス基板上にマレイミド基を導入させることのできる化合物を含む請求項１３６記載の製造方法。

【請求項１３９】　該化合物がＮ－マレイミドカプロイロキシスクシンイミドまたはスクシイミジルー４－（マレイミドフェニル）ブチレートである請求項１３８記載の製造方法。

【請求項１４０】　サンプル中に含まれている可能性のある標的物質に対して特異的に結合するプローブを固相上に互いに独立した複数のスポットとして有するプローブアレイの各々のスポットと該サンプルとを接触させ、該固相上にて該標的物質及び該プローブとの反応物を検出して該サンプル中の該標的物質の有無を検出する方法において、該スポットの各々が、該プローブを含む液体をインクジェット法によって固相上にスポッティングすることによって形成されたものであることを特徴とする標的物質の検出方法。

【請求項１４１】　該標的物質が所定の塩基配列を備えた標的一本鎖核酸であり、該プローブが該塩基配列に対して相補的な塩基配列を有する標的一本鎖核酸プローブである請求項１４０記載の検出方法。

【請求項１４２】　該一本鎖核酸プローブが一本鎖ＤＮＡプローブを含む請求項１４１記載の検出方法。

【請求項１４３】　該一本鎖核酸プローブがＲＮＡプローブを含む請求項１４１記載の検出方法。

【請求項１４４】　該一本鎖核酸プローブが一本鎖ＰＮＡプローブを含む請求項１４１記載の検出方法。

【請求項１４５】　該固相表面と該一本鎖核酸プローブとが各々官能基を有し、該官能基同士は接触によって反応するものである請求項１４１記載の検出方法。

【請求項１４６】　該固相表面が有する官能基がマレイミド基であって、該一本鎖核酸プローブの有する官能基がチオール（ＳＨ）基である請求項１４５記載の検出方法。

【請求項１４７】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とＮ－（６－マレイミドカプロイロキシ）スクシイミドとを反応させて導入したものである請求項１４６記載の検出方法。

【請求項１４８】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とスクシイミジルー４－（マレイミドフェニル）ブチレートとを反応させて導入したものである請求項１４６記載の検出方法。

【請求項１４９】　該ガラス基板上のマレイミド基と該一本鎖核酸のチオール基とを少なくとも３０分反応させる請求項１４６記載の検出方法。

【請求項１５０】　該一本鎖核酸が末端にチオール基を有する一本鎖ＰＮＡプローブを含み、該マレイミド基と該チオール基とを少なくとも２時間以上反応させる請求項１４９記載の検出方法。

【請求項１５１】　該一本鎖ＰＮＡプローブ末端のチオール基が、一本鎖ＰＮＡプローブのＮ末端側へのシステインの結合によって導入されているものである請求項１４６記載の検出方法。

【請求項１５２】　該固相表面が有する官能基がエポキシ基であって、該一本鎖核酸プローブの有する官能基がアミノ基である請求項１４５記載の検出方法。

【請求項１５３】　該固相がガラス板であり、また該エポキシ基は、該ガラス板の表面にエポキシ基を分子内に有するシラン化合物を塗布し、該化合物と該ガラス板とを反応させて導入したものである請求項１５２記載の検出方法。

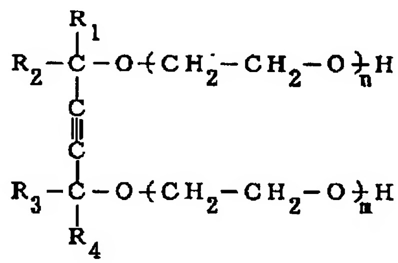
【請求項１５４】　該エポキシ基は、エポキシ基を有するポリグリシジルメタクリレート樹脂の該固相上への塗

布によって導入したものである請求項１５２記載の検出方法。

【請求項１５５】　該液体が、該液体に対して尿素を５～１０ｗｔ％、グリセリンを５～１０ｗｔ％、チオジグリコールを５～１０ｗｔ％、及びアセチレンアルコールを１ｗｔ％含んでいる請求項１４１記載の検出方法。

【請求項１５６】　該アセチレンアルコールが下記一般式（Ｉ）で示される構造を有するものである請求項１５５記載の検出方法。

【化３】



（Ｉ）

（上記式中、R_1、R_2、R_3及びR_4はアルキル基を表わし、ｍおよびｎは夫々整数を表わし、ｍ＝０かつｎ＝０、もしくは１≦ｍ＋ｎ≦３０であって、ｍ＋ｎ＝１の場合はｍまたはｎは０である。）

【請求項１５７】　該液体中の該一本鎖核酸プローブの濃度が０．０５～５００μＭである請求項１５５記載の検出方法。

【請求項１５８】　該液体中の該一本鎖核酸プローブの濃度が２～５０μＭである請求項１５７記載の検出方法。

【請求項１５９】　該一本鎖核酸プローブの長さが２～５０００塩基長である請求項１５５記載の検出方法。

【請求項１６０】　該一本鎖核酸プローブの長さが２～６０塩基長である請求項１５９記載の検出方法。

【請求項１６１】　該インクジェット法がバブルジェット法である請求項１４１記載の検出方法。

【請求項１６２】　該プローブが特定のアミノ酸配列を有するオリゴペプチド或いはポリペプチドである請求項１４０記載の検出方法。

【請求項１６３】　該プローブが蛋白質である請求項１４０記載の検出方法。

【請求項１６４】　該蛋白質が抗体である請求項１６３記載の検出方法。

【請求項１６５】　該蛋白質が酵素である請求項１６３記載の検出方法。

【請求項１６６】　該プローブが抗原である請求項１４０記載の検出方法。

【請求項１６７】　該液体を、該固相上に１平方インチあたり１００００個以上の密度で、互いに独立したスポットとなるようにスポッティングする請求項１４０記載の検出方法。

【請求項１６８】　該固相は表面が平坦であって、且つ

均一な表面特性を有している請求項１４０記載の検出方法。

【請求項１６９】　隣接するスポットの間隔が該スポットの最大幅以上となるようにスポッティングする請求項１６８記載の検出方法。

【請求項１７０】　該固相表面の、スポット以外の部位に核酸が付着しないようにブロッキングされている請求項１６８記載の検出方法。

【請求項１７１】　該ブロッキングが牛血清アルブミンによって達成されている請求項１７０記載の検出方法。

【請求項１７２】　該固相が表面にパターン状に配置されたマトリクスで区画され、パターン状に露出してなる該固相表面を底面とする複数のウェルを備え、該液体を各々のウェルに供給する請求項１４０記載の検出方法。

【請求項１７３】　該固相が光学的に透明であり、該マトリクスが遮光性である請求項１７２記載の検出方法。

【請求項１７４】　該マトリクスが樹脂を含む請求項１７２記載の検出方法。

【請求項１７５】　該マトリクスの表面が疎水性である請求項１７２記載の検出方法。

【請求項１７６】　該ウェルの底面が親水性である請求項１７２記載の検出方法。

【請求項１７７】　該マトリクスの厚さが１～２０μｍである請求項１７２記載の検出方法。

【請求項１７８】　該ウェルの最長幅が２００μｍである請求項１７２記載の検出方法。

【請求項１７９】　該マトリクスの幅が、該ウェルの最長幅の１／２～２倍である請求項１７２記載の検出方法。

【請求項１８０】　試料中に含まれる標的物質の構造を特定する方法であって、固相表面に該特定の物質に対して特異的に結合するプローブのスポットを備えたプローブアレイを用意する工程；該試料を該スポットに接触させる工程；及び該標的物質と該プローブとの結合を検出する工程、を有することを特徴とする標的物質の構造の特定化方法。

【請求項１８１】　該特定の物質が標的一本鎖核酸であり、特定化する構造が該標的一本鎖核酸の塩基配列であり、該プローブアレイは固相上に異なる塩基配列の一本鎖核酸を各々含む複数のスポットを備え、該スポットの少なくとも１つは、該標的一本鎖核酸の予測される塩基配列に対して相補的な塩基配列の一本鎖核酸を含み、且つ該複数のスポットは、各々の一本鎖核酸を含む液体をインクジェット法を用いて該固相上に付着せしめたものである請求項１８０記載の特定化方法。

【請求項１８２】　該一本鎖核酸プローブが一本鎖ＤＮＡプローブを含む請求項１８１記載の特定化方法。

【請求項１８３】　該一本鎖核酸プローブがＲＮＡプローブを含む請求項１８１記載の特定化方法。

【請求項１８４】　該一本鎖核酸プローブが一本鎖ＰＮ

Ａプローブを含む請求項１８１記載の特定化方法。

【請求項１８５】　該固相表面と該一本鎖核酸プローブとが各々官能基を有し、該官能基同士は接触によって反応するものである請求項１８１記載の特定化方法。

【請求項１８６】　該固相表面が有する官能基がマレイミド基であって、該一本鎖核酸プローブの有する官能基がチオール（ＳＨ）基である請求項１８１記載の特定化方法。

【請求項１８７】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とＮ－（６－マレイミドカプロイロキシ）スクシイミドとを反応させて導入したものである請求項１８６記載の特定化方法。

【請求項１８８】　該固相がガラス板であり、また該マレイミド基は、該ガラス板の表面にアミノ基を導入した後、該アミノ基とスクシイミジル－４－（マレイミドフェニル）ブチレートとを反応させて導入したものである請求項１８６記載の特定化方法。

【請求項１８９】　該ガラス基板上のマレイミド基と該一本鎖核酸のチオール基とを少なくとも３０分反応させる請求項１８６記載の特定化方法。

【請求項１９０】　該一本鎖核酸が末端にチオール基を有する一本鎖ＰＮＡプローブを含み、該マレイミド基と該チオール基とを少なくとも２時間以上反応させる請求項１８９記載の特定化方法。

【請求項１９１】　該一本鎖ＰＮＡプローブ末端のチオール基が、一本鎖ＰＮＡプローブのＮ末端側へのシステインの結合によって導入されているものである請求項１８６記載の特定化方法。

【請求項１９２】　該固相表面が有する官能基がエポキシ基であって、該一本鎖核酸プローブの有する官能基がアミノ基である請求項１８５記載の特定化方法。

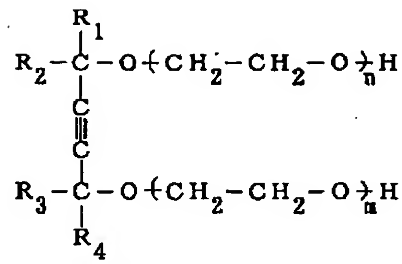
【請求項１９３】　該固相がガラス板であり、また該エポキシ基は、該ガラス板の表面にエポキシ基を分子内に有するシラン化合物を塗布し、該化合物と該ガラス板とを反応させて導入したものである請求項１９２記載の特定化方法。

【請求項１９４】　該エポキシ基は、エポキシ基を有するポリグリシジルメタクリレート樹脂の該固相上への塗布によって導入したものである請求項１９２記載の特定化方法。

【請求項１９５】　該液体が、該液体に対して尿素を５～１０ｗｔ％、グリセリンを５～１０ｗｔ％、チオジグリコールを５～１０ｗｔ％、及びアセチレンアルコールを１ｗｔ％含んでいる請求項１８１記載の特定化方法。

【請求項１９６】　該アセチレンアルコールが下記一般式（Ｉ）で示される構造を有するものである請求項１９５記載の特定化方法。

【化４】



（Ｉ）

（上記式中、R_1、R_2、R_3及びR_4はアルキル基を表わし、ｍおよびｎは夫々整数を表わし、ｍ＝０かつｎ＝０、もしくは１≦ｍ＋ｎ≦３０であって、ｍ＋ｎ＝１の場合はｍまたはｎは０である。）

【請求項１９７】　該液体中の該一本鎖核酸プローブの濃度が０．０５～５００μＭである請求項１９５記載の特定化方法。

【請求項１９８】　該液体中の該一本鎖核酸プローブの濃度が２～５０μＭである請求項１９７記載の特定化方法。

【請求項１９９】　該一本鎖核酸プローブの長さが２～５０００塩基長である請求項１９７記載の特定化方法。

【請求項２００】　該一本鎖核酸プローブの長さが２～６０塩基長である請求項１９９記載の特定化方法。

【請求項２０１】　該インクジェット法がバブルジェット法である請求項１８１記載の特定化方法。

【請求項２０２】　該プローブが特定のアミノ酸配列を有するオリゴペプチド或いはポリペプチドである請求項１８０記載の特定化方法。

【請求項２０３】　該プローブが蛋白質である請求項１８０記載の特定化方法。

【請求項２０４】　該蛋白質が抗体である請求項２０３記載の特定化方法。

【請求項２０５】　該蛋白質が酵素である請求項２０３記載の特定化方法。

【請求項２０６】　該プローブが抗原である請求項１８０記載の特定化方法。

【請求項２０７】　該液体を、該固相上に１平方インチあたり１００００個以上の密度で、互いに独立したスポットとなるようにスポッティングする請求項１８０記載の特定化方法。

【請求項２０８】　該固相は表面が平坦であって、且つ均一な表面特性を有している請求項１８０記載の特定化方法。

【請求項２０９】　隣接するスポットの間隔が該スポットの最大幅以上となるようにスポッティングする請求項２０８記載の特定化方法。

【請求項２１０】　該固相表面の、スポット以外の部位に核酸が付着しないようにブロッキングされている請求項２０８記載の特定化方法。

【請求項２１１】　該ブロッキングが牛血清アルブミンによって達成されている請求項２１０記載の特定化方

法。
【請求項２１２】　該固相が表面にパターン状に配置されたマトリクスで区画され、パターン状に露出してなる該固相表面を底面とする複数のウェルを備え、該液体を各々のウェルに供給する請求項１８０記載の特定化方法。
【請求項２１３】　該固相が光学的に透明であり、該マトリクスが遮光性である請求項２１２記載の特定化方法。
【請求項２１４】　該マトリクスが樹脂を含む請求項２１２記載の特定化方法。
【請求項２１５】　該マトリクスの表面が疎水性である請求項２１２記載の特定化方法。
【請求項２１６】　該ウェルの底面が親水性である請求項２１２記載の特定化方法。
【請求項２１７】　該マトリクスの厚さが１～２０μｍである請求項２１２記載の特定化方法。
【請求項２１８】　該ウェルの最長幅が２００μｍである請求項２１２記載の特定化方法。
【請求項２１９】　該マトリクスの幅が、該ウェルの最長幅の１／２～２倍である請求項２１２記載の特定化方法。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明はプローブを固相にスポッティングする方法、プローブアレイ、その製造方法、そしてプローブアレイを用いた標的一本鎖核酸の検出方法、及び標的一本鎖核酸の塩基配列の特定化方法に関する。
【０００２】
【従来の技術】核酸の塩基配列の決定やサンプル中の標的核酸の検出、各種細菌の同定を迅速、正確に行ない得る技術の一つとして、例えば該標的核酸と特異的に結合し得る物質、いわゆるプローブを固相上に多数並べたプローブアレイの使用が提案されている。
【０００３】このようなプローブアレイの一般的な製造方法としては、例えばヨーロッパ特許第３７３２０３号公報（ＥＰ０３７３２０３Ｂ１）に記載されている様に（１）固相上で核酸プローブを合成していく方法、や（２）予め合成した核酸プローブを固相上に供給する方法等が知られている。上記の（１）の方法の詳細が開示されている先行技術としては例えば米国特許第５４０５７８３号公報（ＵＳＰ５４０５７８３）が挙げられる。
【０００４】また上記（２）の方法としては、例えば米国特許第５６０１９８０号公報（ＵＳＰ５６０１９８０）や「サイエンス（Ｓｃｉｅｎｃｅ）」、第２７０巻、４６７頁、（１９９５）にはマイクロピペッティングを用いてｃＤＮＡをアレイ状に並べる方法が開示されている。
【０００５】ところで上記（１）の方法は、固相上で直接核酸プローブを合成させている為、予め核酸プローブを合成する必要がない。しかし固相上で合成されたプローブ核酸を精製することは困難である。プローブアレイを用いた核酸塩基の配列決定や、サンプル中の標的核酸の検出等の精度は、核酸プローブの塩基配列の精度に大きく依存する。従って上記（１）の方法は、より高品質なプローブアレイの製法としては核酸プローブの精度の向上に更なる改良が求められるところである。
【０００６】一方、上記（２）の方法は、核酸プローブの固相への固定に先立って核酸プローブの合成ステップが必要となる反面、固相への結合に先立って核酸プローブを精製することができる。この理由により現段階においては、より高品質なプローブアレイの製法としては上記（２）の方法は、上記（１）の方法よりも好ましいと考えられる。
【０００７】しかし上記（２）の方法の課題は、核酸プローブを固相に高密度にスポッティングする方法にある。例えばプローブアレイを用いて核酸の塩基配列決定を行なう場合、できる限り多種の核酸プローブを固相上に配置しておくことが好ましい。また遺伝子の変異の検出を効率的に行なう場合には、それぞれの変異に対応した配列を有する核酸プローブを固相上に配置させておく事が好ましい。さらに、サンプル中の標的核酸の検出や、遺伝子の変異、欠損の検出に当たっては、被験者からのサンプルの採取、具体的には血液等の採取はできる限り少量に止めておくことが好ましく、よって少量の検体でできる限り多くの塩基配列の情報を獲得できることが好ましい。これらの点から考えるとプローブアレイには例えば、１インチ角に１００００以上の核酸プローブが配置されていることが好ましい。
【０００８】
【発明が解決しようとする課題】本発明者らはこのような状況の下で種々検討を行なった結果、インクジェット吐出技術を用いて、極めて高密度にプローブをスポッティングすることが出来ることを見出し本発明を為すに至った。
【０００９】そして本発明の目的は、極めて微量のプローブを、該プローブに損傷を与える事なく且つ効率的に固相上に正確にスポッティングする方法を提供することにある。
【００１０】また本発明の他の目的は、少量の検体からでも核酸に関するより多くの情報をより正確に検査可能なプローブアレイを提供することにある。
【００１１】また本発明の更に他の目的は、プローブが固相上に多数結合しているプローブアレイを、プローブを損傷することなく、また効率良く製造する方法を提供することにある。
【００１２】更に本発明の他の目的は、サンプル中に含まれている可能性のある標的物質を効率的に検出する方法を提供することにある。

【００１３】更にまた本発明の他の目的は、少量の検体からでも標的物質の構造に関する情報を獲得可能な標的物質の構造の特定化方法を提供することにある。

【００１４】

【課題を解決するための手段】上記の目的を達成することのできる、本発明の一実施態様にかかるスポッティング方法は、標的物質に対して特異的に結合可能であるプローブを含む液体をインクジェット法により固相表面に供給し、該固相表面に付着させる工程を有することを特徴とするものである。

【００１５】上記態様にかかるスポッティング方法を用いることによってプローブを固相上に正確に、且つ効率的に付与することができ、プローブアレイを効率的に製造することが出来るものである。

【００１６】また本発明の一実施態様にかかるプローブアレイは、固相表面の複数の部位に互いに独立しているプローブのスポットを１平方インチ内に１００００個以上の密度で備えていることを特徴とするものである。上記態様にかかるプローブアレイによればスポットを極めて高密度に有していることから、少量の検体からでも多くの情報を得ることができる。

【００１７】また本発明の一実施態様にかかるプローブアレイの製造方法は、固相表面の複数の箇所に独立して、標的物質に対して特異的に結合可能であるプローブを含むスポットを有するプローブアレイの製造方法であって、該プローブを含む液体をインクジェット法を用いて該固相表面の所定の位置に供給し、付着させる工程を有することを特徴とするものである。この態様によればプローブを損なうことなしにスポットが高密度に配置されたプローブアレイを効率的に製造することができるものである。

【００１８】また上記の目的を達成することのできる、本発明の一実施態様にかかる標的物質の検出方法は、サンプル中に含まれている可能性のある標的物質に対して特異的に結合するプローブを固相上に互いに独立した複数のスポットとして有するプローブアレイの各々のスポットと該サンプルとを接触させ、該固相上にて該標的物質及びプローブとの反応物を検出して該サンプル中の該標的物質の有無を検出する方法において、該スポットの各々が、該プローブを含む液体をインクジェット法によって固相上にスポッティングすることによって形成されたものであることを特徴とするものである。この態様によれば標的物質を効率的に検出することができるものである。

【００１９】更に上記の目的を達成することのできる、本発明の一実施態様にかかる標的物質の構造の特定化方法は、試料中に含まれる標的物質の構造を特定する方法であって、固相表面に該特定の物質に対して特異的に結合するプローブのスポットを備えたプローブアレイを用意する工程；該試料を該スポットに接触させる工程；及び該標的物質と該プローブとの結合を検出する工程、を有することを特徴とするものである。この態様を用いることによって少量の検体からでも該検体中の標的物質の構造、例えば標的物質が一本鎖核酸の場合にはその塩基配列の特定を効率的に行なうことができるものである。

【００２０】なおＵＳＰ５６０１９８０号公報には核酸プローブのスポッティングにはコンベンショナルなインクジェット技術を用いることは適当でないと認定されている。即ちＵＳＰ５６０１９８０号公報の第２欄の第３１行～第５２行目には、圧力波（pressure wave）によって少量のインクを吐出させるインクジェットプリンタ技術の利用が適当でないと記載され、その理由としてインク吐出の為の圧力波がインク温度の急激な温度上昇を招き、核酸プローブに損傷を与える可能性が有り、また吐出時のインクの飛び散りが隣接する核酸プローブのスポット同士のコンタミネーションを引き起こす危険性を挙げている。その上でＵＳＰ５６０１９８０号公報においては、ガス圧を利用してマイクロピペットの先端に核酸プローブを含む液体の滴を、該滴のサイズをモニターしつつ形成させ、所定のサイズに達した時点で圧力印加を止め、該滴を固相上に供給してプローブアレイを製造する方法を開示している。

【００２１】またＵＳＰ５４７４７９６号公報には、固相表面に疎水性及び親水性のマトリクスを形成し、その親水性部分に４種類の塩基をピエゾエレクトリックインパルスジェットポンプ装置（Piezoelectric Impulse Jet Pump Apparatus）を用いて順次吐出せしめて、オリゴヌクレオチドアレイを製造すること、そしてそれを用いて標的核酸の塩基配列を決定する方法が開示されている。

【００２２】しかしこれらの先行技術には、予め所定の長さの塩基配列を有する核酸プローブをインクジェット技術を用いて吐出させて、核酸プローブを高密度に、且つ正確に配列せしめる技術については何ら開示されていない。

【００２３】

【発明の実施の形態】（プローブアレイ製法概略）図１及び図２は本発明に係るプローブアレイ、例えば核酸プローブアレイの製造方法の概略説明図である。図１において１０１は吐出液としてのプローブ、例えば核酸プローブを含む液体を吐出可能に保持している液体供給系（ノズル）、１０３は該核酸プローブが結合されるべき固相（例えば透明ガラス板等）、１０５はインクジェットヘッドの一種である、該液体に熱エネルギーを付与して吐出させる機構を備えるバブルジェットヘッドである。１０４はバブルジェットヘッド１０５から吐出された核酸プローブを含む液体である。また図２は、図１のバブルジェットヘッド１０５のＡ－Ａ線断面図であり、図２において１０５はバブルジェットヘッド、１０７は吐出されるべき核酸プローブを含む液体、そして１１７

は該液体に吐出エネルギーを付与する発熱部を有する基板部分である。基板部分１１７は、酸化シリコン等で形成されている保護膜１０９、アルミニウム等で形成されている電極１１１－１、１１１－２、ニクロム等で形成されている発熱抵抗体層１１３、蓄熱層１１５及び放熱性の良好なアルミナ等で形成されている支持体１１６を含んでいる。

【００２４】核酸プローブを含む液体１０７は吐出オリフィス（吐出口）１１９まできており、所定の圧力によってメニスカス１２１を形成している。ここで電極１１１－１、１１１－２に電気信号が加わると、１２３で示す領域（発泡領域）が急激に発熱し、ここに接している液体１０７に気泡が発生し、その圧力でメニスカスが吐出し、オリフィス１１９から液体１０７が吐出し、固相１０３の表面に向って飛翔する。このような構造を備えるバブルジェットヘッドを用いて吐出可能な液体の量は、そのノズルのサイズ等によって異なるが、例えば４～５０ピコリットル程度に制御することが可能であり、高密度に核酸プローブを配置させる手段として極めて有効である。

（吐出液と固相の関係）

（固相上でのスポット直径）核酸プローブの固相上での密度を上記した様な値（例えば1インチ各に１００００個以上、上限としては1×10^{6}個程度）にするためには各々の核酸プローブのスポット径は、例えば２０～１００μm程度であることが好ましく、また互いのスポットが互いに独立していることが好ましい。そしてこのようなスポットは、バブルジェットヘッドから吐出される液体の特性、及び該液体が付着する固相の表面特性等によって決定される。

【００２５】（吐出液の特性）吐出用の液体としては、バブルジェットヘッドから吐出可能であって、且つヘッドから吐出された該液体が固相上の所望の位置に着弾し、更に核酸プローブとの混合状態、及び吐出時において該核酸プローブが損傷を受けなければいかなる液体でも用いることができる。

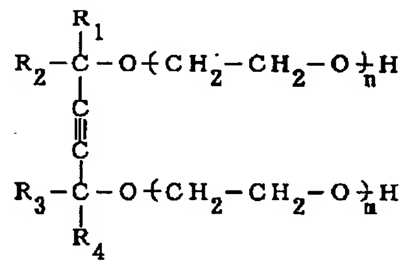
【００２６】そしてバブルジェットヘッドからの吐出性という観点からは、該液体の特性としては例えば、その粘度が１～１５ｃｐｓ、表面張力が３０ｄｙｎ／ｃm以上が好ましい。また粘度を１～５ｃｐｓ、表面張力を３０～５０ｄｙｎ／ｃmとした場合、固相上での着弾位置が極めて正確なものとなり特に好適に用いられる。

【００２７】次に該液体のインクジェット吐出特性、及び液体中及びバブルジェット吐出時の核酸プローブの安定性を考慮すると、液体中には例えば2ｍｅｒ～５０００ｍｅｒ、特には2ｍｅｒ～６０ｍｅｒの核酸プローブを、０．０５～５００μM、特には２～５０μMの濃度で含有させることが好ましい。

【００２８】（吐出液組成）バブルジェットヘッドから吐出される液体の組成としては、上記した様に核酸プローブと混合したとき、及びバブルジェットヘッドから吐出させたときに核酸プローブに対して影響を実質的に与えないものであって、且つバブルジェットヘッドを用いて固相に対して正常に吐出可能である液体組成が好ましい条件を満たせば、特に限定されるものでないが、例えばグリセリン、尿素、チオジグリコール又はエチレングリコール、イソプロピルアルコール及び下記式（Ｉ）で示されるアセチレンアルコールを含む液体は好ましいものである。

【００２９】

【化５】



（Ｉ）

【００３０】（上記式（Ｉ）中、R_1、R_2、R_3及びR_4はアルキル基、具体的には例えば炭素数１～４の直鎖状または分岐鎖状のアルキル基を表わし、m及びｎは各々整数を表わし、ｍ＝０且つｎ＝０、もしくは$1\leqq m+n\leqq 30$であって、ｍ＋ｎ＝１の場合はmまたはｎは０である。）

更に具体的には尿素を５～１０ｗｔ％、グリセリンを５～１０ｗｔ％、チオジグリコールを５～１０ｗｔ％、及び上記式（Ｉ）で示されるアセチレンアルコールを０．０２～５ｗｔ％、より好ましくは０．５～１ｗｔ％を含む液体が好適に用いられる。

【００３１】この液体を用いた場合、バブルジェットヘッドから核酸プローブを含む液体を吐出させて固相上に付着させたときのスポットの形状が円形で、また吐出された範囲が広がることがなく、高密度に核酸プローブをスポッティングした場合にも、隣接するスポットとの連結を有効に抑えることができる。更に固相上にスポッティングされた核酸プローブの変質も認められない。なお本発明の核酸プローブアレイの製造に用いる液体の特性は上記のものに限定されるものではない。例えば固相表面に、バブルジェットヘッドで固相上に付与した液体が、該固相上で広がり、そして隣接するスポットとの間で混合してしまうのを防ぐような、ウェルのような構造を設けた場合には、液体の粘度や表面張力、更には核酸プローブの塩基長や濃度も上記の範囲外であっても用いることができる。

【００３２】（固相と核酸の官能基の種類）固相上に付着せしめた核酸プローブのスポットを更に限定された位置に止めさせ、隣接するスポットとのコンタミネーションをより確実に防ぐ為に有効であり、かつ核酸プローブを固相上に強固に結合させるのに有効な手段として、核

酸プローブと固相との双方に互いに反応可能な官能基を結合させる方法が挙げられる。

【0033】（SH基とマレイミド基）好ましい例としては例えば、マレイミド基とチオール（－SH）基との組合わせを用いる例が挙げられる。即ち核酸プローブの末端にチオール（－SH）基を結合させておき、固相表面がマレイミド基を有するように処理しておくことで、固相表面に供給された核酸プローブのチオール基と固相表面のマレイミド基とが反応して核酸プローブを固定化し、その結果核酸プローブのスポットを固相上の所定の位置に形成することができる。特に末端にチオール基を有する核酸プローブを上記した組成の液体に溶解させたものをバブルジェットヘッドを用いてマレイミド基を導入した固相表面に付与した場合、核酸プローブ溶液は固相上に極めて小さなスポットを形成する。その結果、核酸プローブの小さなスポットを固相表面の所定の位置に形成することができる。この場合、固相表面に例えば親水性及び疎水性のマトリクスからなるウェルを形成し、スポット間の連結を防止する様な構成を予め設けておく必要性は認められない。

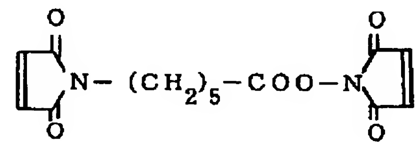
【0034】例えば塩基長１８ｍｅｒの核酸プローブを濃度８μＭで含む、粘度や表面張力が前記した範囲内となるように調整した液体を、バブルジェットプリンタ（商品名：ＢＪＣ６２０；キヤノン（株）社製、但し平板に印字可能に改造したもの）を用いて、固相とバブルジェットヘッドのノズルの間隔を１．２～１．５mm程度に設定し、該ノズルから吐出させた場合（吐出量は約２４ピコリットル）、固相上には直径約７０～１００μm程度のスポットを形成することができ、また液体が固相表面に着弾したときの飛沫に由来するスポット（以降「サテライトスポット」と称する）は目視では全く認められなかった。該固相上のマレイミド基と核酸プローブ末端のＳＨ基との反応は、吐出される液体の条件にもよるが、室温（２５℃）下で３０分程度で完了する。なおこの時間は液体の吐出にピエゾジェットヘッドを用いた場合と比較して短い。その理由は明らかでないが、バブルジェット法ではその原理によりヘッド内の核酸プローブを含む液体の温度が上昇し、その結果マレイミド基とチオール基の反応効率が上昇して反応時間が短縮されるものと考えられる。

【0035】なお、マレイミド基とチオール基との組合せを用いる場合、核酸プローブを含む溶液にチオジグリコールを含有させることが好ましい。チオール基は中性及び弱アルカリ性条件下ではジスルフィド結合（－Ｓ－Ｓ－）を形成し、二量体をなることがある。しかし、チオジグリコールを加えることで、二量体形成によるチオール基とマレイミド基との反応性の低下を防ぐことができる。

【0036】固相表面へのマレイミド基の導入方法としては、種々の方法が利用できるが、例えばガラス基板にアミノシランカップリング剤を反応させ、次にそのアミノ基と下記構造式で示されるＮ－（６－マレイミドカプロイロキシ）スクシイミド（N-(6-Maleimidocaproyloxy) succinimide）を含む試薬（ＥＭＣＳ試薬：Ｄｏｊｉｎ社製）とを反応させることで可能である。

【0037】

【化６】



【0038】またチオール基が結合した核酸プローブは、例えばＤＮＡ自動合成機を用いてＤＮＡを自動合成する際に５’末端の試薬として５’－Ｔｈｉｏｌ－ＭｏｄｉｆｉｅｒＣ６（Glen Research社製）を用いる事により合成することができ、通常の脱保護反応の後、高速液体クロマトグラフィーにより精製することで得られる。

【0039】（アミノ基とエポキシ基）固定化に利用する官能基の組合わせとしては、上記したチオール基とマレイミド基の組合わせ以外にも、例えばエポキシ基（固相上）とアミノ基（核酸プローブ末端）の組合わせ等が挙げられる。固相表面へのエポキシ基の導入は、例えばエポキシ基を有するポリグリシジルメタクリレートを樹脂からなる固相表面に塗布したり、エポキシ基を有するシランカップリング剤をガラス製の固相表面に塗布してガラスと反応させる方法等が挙げられる。

【0040】上記したように固相表面と一本鎖核酸プローブの末端とに互いに反応して共有結合を形成するような官能基を導入した場合、該核酸プローブと固相とがより強固に結合される。また該核酸プローブの固相との結合部位を常に末端とすることができる為、各々のスポットでの核酸プローブの状態を均一にすることができる。その結果各々のスポットにおける核酸プローブと標的核酸とのハイブリダイゼーションの条件が揃うこととなり、より一層精度の向上した標的核酸の検出や塩基配列の特定が可能となるものと考えられる。更に末端に官能基のついた核酸プローブと固相とを共有結合させる事は、非共有結合（例えば静電的な結合等）によって固相上に固定した核酸プローブに比べ、配列や長さの違いによるプローブＤＮＡの結合量の差を生じることなく定量的にプローブアレイを作製できる。更にまた核酸の塩基配列部分が全てハイブリダイゼーション反応に寄与する為、ハイブリダイゼーション反応の効率を著しく上昇させる事ができる。また一本鎖核酸プローブの標的核酸とのハイブリダイゼーションに関与する部分と固相との反応に関与する官能基との間にリンカー部分として例えば炭素数１～７程度のアルキレン基を導入しておいても良い。これによって固相に核酸プローブを結合させたとき

に該固相表面と該核酸プローブとの間に所定の距離を設けることができ、核酸プローブと標的核酸との反応効率のより一層の向上が期待できる。

【0041】（アレイの製法）次に本発明に係るプローブアレイの製造方法の、現状における最も好ましい態様の一つについて説明する。まず核酸プローブを分散させる液体としてグリセリン７．５ｗｔ％、尿素７．５ｗｔ％、チオジグリコール７．５ｗｔ％、上記一般式（Ｉ）で示される構造のアセチレンアルコール（例えば商品名：アセチレノールＥＨ；川研ファインケミカル（株）社製）１ｗｔ％を含む液体を用意する。次に末端にチオール基が結合している、長さが例えば２～５０００ｍｅｒ程度、特には２～６０ｍｅｒ程度の一本鎖核酸プローブをＤＮＡ自動合成機を用いて合成する。次いでこの核酸プローブを上記液体に、例えば濃度が０．０５～５００μＭ、特には２～５０μＭの範囲で、該液体の粘度が１～１５ｃｐｓ、特には１～５ｃｐｓ、また表面張力が３０ｄｙｎ／ｃｍ以上、特には３０～５０ｄｙｎ／ｃｍとなるように混合し、吐出用の液体とする。そしてこの吐出用液体を例えばバブルジェットヘッドのノズル内に充填する。また固相には上記の方法に従って表面にマレイミド基を導入しておく。そして該固相と該バブルジェットヘッドを、該固相のマレイミド基が結合している面とバブルジェットヘッドのノズル面との距離が１．２～１．５ｍｍ程度にまで近接させ、該バブルジェットヘッドを駆動させて該液体を吐出させる。ここで吐出条件としては固相上のスポットが互いに連結することがないような印字パターンに設定することが好ましい。例えばスポッティングに用いるバブルジェットヘッドの解像度が３６０×７２０ｄｐｉの場合には、３６０ｄｐｉの方向には１回吐出後２回空吐出させ、７２０ｄｐｉの方向には１回吐出後５回空吐出させるという条件でスポッティングした場合、各々のスポット間のスペースは約１００μｍとなり、隣接するスポットとのコンタミネーションを十分に防ぐことが可能である。

【0042】次いで固相上のマレイミド基と液体中の核酸プローブのチオール基の反応が進み、該核酸プローブが固相に確実に固定されるまで該固相を例えば加湿チャンバー内に静置する。この時間は上記したように例えば室温（約２５℃）で３０分程度で十分である。その後固相上にあって未反応の核酸プローブを洗い流して核酸プローブアレイが得られる。

【0043】ここでこの核酸プローブアレイを用いて、例えば標的核酸の検出等を行なう場合の検出精度（Ｓ／Ｎ比）の向上を図ることを目的として、該核酸プローブを固相表面に固定した後、該固相の核酸プローブ非結合部分がサンプル中に含まれる標的核酸等と結合しないようにブロッキングを行なうことが好ましい。ブロッキングは例えば、該固相を２％ウシ血清アルブミン水溶液中に、例えば２時間程度浸したり、固相表面の核酸プローブと結合していないマレイミド基を分解させることによって可能である。例えばＤＴＴ（ジチオスレイトール）、β－メルカプトエタノール等を用いても可能である。しかし、標識ＤＮＡの吸着を防ぐ効果からすると、ウシ血清アルブミン水溶液が最も適する。尚このブロッキングの工程は必要に応じて行なえば良く、例えばサンプルの該プローブアレイへの供給を各々のスポットに対して限定的に行ない、スポット以外の部位へのサンプルの付着が実質的にない場合には行なわなくても良い。また固相に予めウェルが形成され、そのウェル以外の部分が核酸プローブが付着し難い様に加工されている場合にもブロッキングの工程を省略することができる。

【0044】この様にして作製するプローブアレイはその用途に応じて、例えば同じ核酸プローブを含む複数のスポットを有するように構成してもよく、また異種の核酸プローブを各々含む複数のスポットを有する様に構成してもよい。そしてこの様な方法によって核酸プローブが高密度に配置されたプローブアレイは、その後標的一本鎖核酸の検出や、塩基配列の特定等に用いられる。例えばサンプル中に含まれている可能性のある、塩基配列が既知の標的一本鎖核酸の検出に用いる場合には、該標的一本鎖核酸の塩基配列に対して相補的な塩基配列を有する一本鎖核酸をプローブとして用い、該プローブを含む複数のスポットが固相上に配置されているプローブアレイを用意し、該プローブアレイの各々のスポットに、サンプルを供給して該標的一本鎖核酸と核酸プローブとがハイブリダイズするような条件下に置いた後、各々のスポットにおけるハイブリッドの有無を蛍光検出等の既知の方法で検出する。それによってサンプル中の標的物質の有無の検出を行なうことができる。またサンプル中に含まれている標的一本鎖核酸の塩基配列の特定に用いる場合には、該標的一本鎖核酸の塩基配列の複数の候補を設定し、該塩基配列群に対して各々相補的な塩基配列を有する一本鎖核酸をプローブとして該固相にスポッティングする。次いで各々のスポットにサンプルを供給して該標的一本鎖核酸と核酸プローブとがハイブリダイズするような条件下に置いた後、各々のスポットにおけるハイブリッドの有無を蛍光検出等の既知の方法で検出する。これにより標的一本鎖核酸の塩基配列の特定を行なうことができる。また本発明に係わるプローブアレイの他の用途としては、例えばＤＮＡ結合蛋白質が認識する特異的な塩基配列のスクリーニングやＤＮＡに結合する性質を有する化学物質のスクリーニングへの適用が考えられる。

【0045】（インクジェットヘッドの種類）なお上記の説明においては、核酸プローブの固相への付与をバブルジェットヘッドで行なう構成のみを説明したが、本発明においてはピエゾ素子の振動圧を利用してノズル内の液体を吐出せしめるピエゾジェットヘッドを用いることも可能である。しかし前記した様にバブルジェットヘッ

25　　　　　　　　　　　　　　　　26

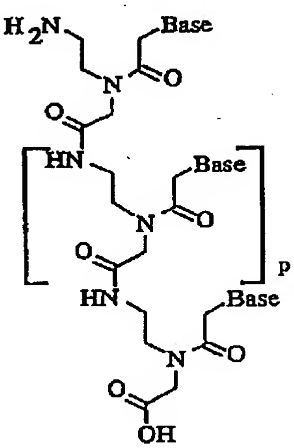
ドを用いた場合、固相への結合反応が短時間で完了し、またＤＮＡの二次構造も熱により解消されるため、次に続くハイブリダイゼーション反応の効率をも上昇させることができるという点で、バブルジェットヘッドは本発明にとってより好適に用いられるインクジェットヘッドである。

【００４６】更に２以上のスポット間で含有される核酸プローブが異なる様に複数のヘッドを備えたインクジェットヘッドを用いて複数のスポットを同時に固相上に形成しても良い。

（ＰＮＡ／ＤＮＡ）ここまで、プローブの一例として核酸プローブを用いて本発明を説明した。核酸プローブの例としては、デオキシリボ核酸（ＤＮＡ）プローブ、リボ核酸（ＲＮＡ）プローブ及びペプチド核酸（ＰＮＡ）プローブを含むものである。ＰＮＡはＤＮＡに含まれる４種の塩基（アデニン、グアニン、チミン、シトシン）が糖ーリン酸エステル主鎖ではなくてペプチド主鎖に結合し、下記式（ＩＩ）に示される様な構造を有する合成オリゴヌクレオチドである。

【００４７】

【化７】



(II)

【００４８】（式中「Ｂａｓｅ」はＤＮＡを構成する４種類の塩基（アデニン、シトシン、チミン、グアニン）の何れかを示す。またｐはＰＮＡの塩基長を表わす。）

ＰＮＡは、例えばｔＢＯＣ型固相合成法やＦｍｏｃ型固相合成法として知られている方法によって合成することができる。そしてＰＮＡはＤＮＡやＲＮＡ等の天然のオリゴヌクレオチドと比較してヌクレアーゼやプロテアーゼ等の酵素に対する強い耐性を有し、血清中でも酵素的開裂が殆ど、若しくは全く起らず安定である。また糖部やリン酸基を有していない為、溶液のイオン強度の影響を殆ど受けず、従ってＰＮＡと標的一本鎖核酸とを反応させる際の塩濃度等の調整を行なう必要がなく、更には静電的な反発が無いためにＤＮＡプローブと標的一本鎖核酸とのハイブリッドやＲＮＡプローブと標的一本鎖核酸とのハイブリッドと比較してＰＮＡと標的一本鎖核酸とのハイブリッドのほうが熱安定性に優れているとも考えられている。そしてこれらの特性から標的核酸の検出や塩基配列の決定に用いるプローブとして有望なものである。そして前記した本発明に係る核酸プローブアレイの製造方法は、核酸プローブとしてＰＮＡプローブを適用した場合にも有効であり、ＰＮＡプローブが高密度に、且つ高精度に配置されたＰＮＡプローブアレイを容易に製造することができる。具体的には、例えばＰＮＡプローブを固相上の限定された位置に止めさせてプローブアレイの高密度化を図る方法としてはＤＮＡプローブやＲＮＡプローブと同様に、ＰＮＡプローブの末端と固相表面との各々に互いに反応性を有する官能基を導入する方法を用いることができ、反応性の基の好ましい組合わせの一つは上述したのと同様のチオール基（ＰＮＡ末端）とマレイミド基（固相表面）の組合わせである。ＰＮＡ末端へのチオール基の導入は、例えばＰＮＡプローブのＮ末端（ＤＮＡの５’末端に相当）にチオール基を含むシステイン（$CH(NH_2)(COOH)CH_2SH$）基等を導入することで達成される。ＰＮＡプローブのＮ末端へのシステインの導入は、例えばＰＮＡプローブのＮ末端のアミノ基とシステインのカルボキシル基を反応させることによって行なうことができる。またＰＮＡプローブのＮ末端のアミノ基と例えば$N_2H(CH_2)_2O(CH_2)_2OCH_2COOH$のようにアミノ基及びカルボキシル基を有している様な適当なリンカーのカルボキシル基とを反応させ、次いで該リンカーのアミノ基とシステインのカルボキシル基とを反応させることでリンカーを介してＰＮＡプローブのＮ末端にシステインを結合させることもできる。この様にリンカーを介して固相との結合基を導入した場合、ＰＮＡプローブの標的物質との反応部位を固相から所定の距離だけ離間させることができ、ハイブリダイゼーション効率のより一層の向上が期待される。

【００４９】またＰＮＡはその塩基長によっては水に対する溶解性が同じ塩基長のＤＮＡと比較すると低い場合があり、インクジェット吐出用の液体を調製する際にはＰＮＡを予めトリフルオロ酢酸（例えば０．１ｗｔ％トリフルオロ酢酸水溶液等）等に溶解させた後、前記した種々の溶媒を用いてインクジェット吐出に適合する特性に調製することが好ましい。特にトリフルオロ酢酸に溶解させておくことは、ＰＮＡ末端のシステイン残基中のチオール基の酸化によるシスチンへの変性を防ぎ、ＰＮＡのチオール基と固相表面のマレイミド基との反応効率のより一層の向上を図る上で好ましい。またＤＮＡプローブやＲＮＡプローブの末端に導入したチオール基と固相表面のマレイミド基の反応時間は前記した様に３０分（バブルジェットヘッドを用いた場合）で十分であるが、ＰＮＡの場合にはバブルジェットヘッドを用いた場合であっても２時間程度反応させることが好ましい。

【００５０】更にプローブとしては核酸プローブに限定されず、検出・分析対象となるサンプル中の標的物質と特異的に結合し得る物質、例えばレセプターと特異的に

結合可能なリガンド、リガンドと特異的に結合可能なレセプター、特定のアミノ酸配列を有するオリゴペプチドまたはポリペプチドと結合可能なオリゴペプチドやポリペプチド、更にはタンパク質（例えば抗体、抗原、酵素等）等をプローブとして用いることができる。この場合、いずれも蛋白質に含まれているシステイン残基のSH基を反応に利用することができる。

【0051】以上説明した様にインクジェット吐出プロセスを用いてプローブ溶液を固相に供給する工程を含むプローブアレイの製造方法によれば、プローブアレイを極めて容易に形成することができる。特に核酸プローブと固相表面との間で共有結合が形成される様に各々に官能基を導入した場合には、固相表面に予めウェル等を有しない、即ち実質的に平坦で且つ表面特性（水に対する濡れ易さ等）が均一な固相を用いても隣接するスポット同士が連結してしまうことがない。その結果核酸プローブが精度良く、且つ高密度に配列された核酸プローブアレイを極めて効率的に、しかも低コストで製造することができる。

【0052】なおこのことは本発明において表面にウェルを備えた固相を用いることを何ら排除するものではない。例えばプローブ溶液が供給されるウェルの間に光不透過性のマトリクスパターン（以降「ブラックマトリクス」と称する）を予め形成しておいた場合、固相上でのプローブと標的物質とのハイブリダイゼイションを光学的に検出（例えば蛍光の検出）する様な場合の検出精度（SN比）をより一層向上させることができる。また隣接するウェルの間に、表面がプローブ溶液に対する親和性の低いマトリクスを設けておいた場合、プローブ溶液のウェルへの供給にあたって多少の位置ずれが生じたとしても所望のウェルにスムーズにプローブ溶液を供給することができる。このような効果を利用することを目的として表面にウェルを備えた固相を用いてもよい。以下に表面にマトリクスを有する固相、その製造方法及び該固相の本実施態様における使用方法について説明する。

【0053】図5に、本態様におけるプローブアレイの一例を示す。図5（A）は平面図であり、図5（B）はそのBB断面図である。このプローブアレイは、固相103上にマトリクス状に配置された凹部（ウェル）127を形成した枠体構造を有するマトリクスパターン125を設けた構造を有する。マトリクス125（凸部）によって互いに隔離されたウェル127は、マトリクスパターン中の貫通孔（くり抜き部）として設けられたもので、その側壁は凸部からなり、その底面129には固相103の表面が露出した状態にある。固相103の表面露出部分は、プローブと結合可能な表面を形成しており、所定の凹部にプローブ（不図示）が固定されている。

【0054】マトリクスパターンを形成する材料は、プローブと標的物質との反応物を光学的に検出、例えば、反応物の発する蛍光を測定して検出する方法を用いる場合には、検出感度、S／N比及び信頼性の向上を考慮すると遮光性を有するものが望ましい。そのような材料としては、例えば金属（クロム、アルミ、金等）及び黒色の樹脂等が挙げられる。該黒色の樹脂としては、アクリル、ポリカーボネート、ポリスチレン、ポリエチレン、ポリイミド、アクリル酸モノマー、ウレタンアクリレート等の樹脂や、フォトレジスト等の感光性の樹脂に黒色の染料や黒色の顔料を含有させたものが挙げられる。感光性樹脂の具体例としては、例えばUVレジスト、DEEP－UVレジスト、紫外線硬化樹脂等を用いることができる。UVレジストとしては、環化ポリイソプレン－芳香族ビスアジド系レジスト、フェノール樹脂－芳香族アジド化合物系レジスト等のネガレジスト、ノボラック樹脂－ジアゾナフトキノン系レジスト等のポジレジストを挙げることができる。

【0055】DEEP－UVレジストとしては、ポジ型レジストとして、例えば、ポリメチルメタクリレート、ポリメチレンスルホン、ポリヘキサフルオロブチルメタクリレート、ポリメチルイソプロペニルケトン、および、臭化ポリ1－トリメチルシリルプロピン等の放射線分解型ポリマーレジスト、コール酸o－ニトロベンジルエステル等の溶解抑制剤系レジスト等を挙げることができ、ネガ型レジストとして、ポリビニルフェノール－3，3'－ジアジドジフェニルスルホン、および、ポリメタクリル酸グリシジル等を挙げることができる。

【0056】紫外線硬化樹脂としては、ベンゾフェノン、および、その置換誘導体、ベンゾイン、および、その置換誘導体、アセトフェノン、および、その置換誘導体、ベンジル等のオキシム系化合物等のなかから選ばれる、1種、または、2種以上の光重合開始剤を2～10重量%程度含有した、ポリエステルアクリレート、エポキシアクリレート、および、ウレタンジアクリレート等を挙げることができる。黒色の顔料としては、カーボンブラックや黒色有機顔料を用いることができる。

【0057】なお、プローブと標的物質の反応物の検出を、光学的に行なわない場合や、マトリクスからの光が反応物の光学的検出に影響を与えない場合には、マトリクスパターン形成材料として非遮光性の物を用いる事は何ら妨げられるものではない。

【0058】次に上記した様な材料を用いてマトリクスパターンを形成する一つの方法としては基板表面にコートした樹脂や金属上にフォトレジストをコートしパターニングの後に樹脂をエッチング等の工程によりパターニングする方法が挙げられる。また、感光性の樹脂であれば、樹脂そのものをフォトマスクを用いたフォトリソグラフィーのプロセスにより露光、現像、必要に応じて硬化することによりパターニングすることも可能である。

【0059】ここでマトリクス125を樹脂製とした場合、マトリクス125の表面は疎水性となる。この構成

はウェルに供給するプローブを含む溶液として水系の溶液を用いる場合に好ましいものである。即ちウェルにプローブ溶液をインクジェット法を用いて供給する際に多少の位置ずれを伴ってプローブ溶液が供給されたとしても、所望のウェルにプローブ溶液が極めてスムーズに供給されることになる。また、同時に隣接するウェル間で、異なる種類のプローブを供給した場合でも、これらウエル間に供給された異なるプローブ溶液間での交じり合い（クロスコンタミネーション）を防ぐことも可能となる。

【００６０】通常、ペプチド、核酸等の生体関連物質のプローブ溶液は水系の溶液であることが多いので、このような場合にはマトリクスパターンが撥水性となるこの構成を好適に利用できる。

【００６１】次にウェルの底面（固相表面の露出部）をプローブと結合可能な構成とする方法について説明する。ウェルの底面に保持させる官能基は、プローブに担持させる官能基との組合わせによって異なる。例えばプローブとして末端にチオール基を導入した核酸プローブを用いる場合には、前述したように固相表面にマレイミド基を導入しておくことでウェルに供給した核酸プローブのチオール基は固相表面のマレイミド基と共有結合を形成し、核酸プローブが固相表面に固定される。同様にアミノ基を核酸プローブ末端に有する核酸プローブに対しては固相表面へのエポキシ基の導入が好ましい。この様な官能基の他の組合わせとしては、例えばカルボキシル基（スクシンイミド誘導体の核酸プローブ末端への導入による）を末端に有する核酸プローブに対しては固相表面へのアミノ基の導入が好ましい。このアミノ基とエポキシ基の組合わせは、チオール基とマレイミド基の組合わせと比較すると核酸プローブ溶液をインクジェット吐出方法で吐出した際の固相上への定着性は良好でないが、固相にウェルを設けてある場合には無視し得る程度のものである。

【００６２】アミノ基やエポキシ基の固相表面への導入は、前述した様に固相としてガラス板を用いる場合には、まず水酸化カリウムや水酸化ナトリウム等のアルカリで該ガラス板表面を処理して水酸基（シラノール基）を表面に露出させた後、アミノ基を導入したシラン化合物（例えばＮ－β－（アミノエチル）－γ－アミノプロピルトリメトキシシラン等）やエポキシ基を導入したシラン化合物（例えばγ－グリシドキシプロピルトリメトキシシラン等）を含むシランカップリング剤を該ガラス板表面の水酸基と反応させることによって行なうことができる。またマレイミド基は、上記の方法によってガラス板表面にアミノ基を導入した後にＮ－マレイミドカプロイロキシスクシンイミドやスクシイミジルー４－（マレイミドフェニル）ブチレート等を該アミノ基と反応させることでガラス板表面に導入することができる。

【００６３】なおＮ－β－（アミノエチル）－γ－アミノプロピルトリメトキシシラン、γ－グリシドキシプロピルトリメトキシシラン及びスクシイミジルー４－（マレイミドフェニル）ブチレートの構造は下記の通りである。

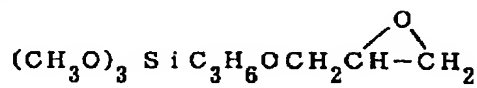
①Ｎ－β（アミノエチル）－γ－アミノプロピルトリメトキシシラン：

$(CH_3O)_2SiC_3H_6NHC_2H_4NH_2$

②γ－グリシドキシプロピルトリメトキシシラン：

【００６４】

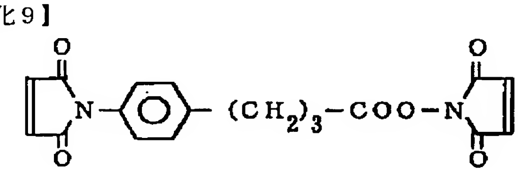
【化８】



【００６５】③スクシイミジルー４－（マレイミドフェニル）ブチレート：

【００６６】

【化９】



【００６７】上記した固相の表面処理においてエポキシ基を固相表面に導入した場合、該エポキシ基とプローブとを結合させた後、未反応のエポキシ基をエタノールアミン水溶液等を用いて開環させて水酸基に変えることにより、ウェルの底面を親水性にすることができる。この操作はプローブを結合させたウェルに該プローブと特異的に反応する標的物質を含む水系溶媒を供給する場合に好ましいものである。

【００６８】また固相として樹脂基板を用いる場合、例えばOrganic Thin Films and Surface, Vol.20, Academic Pressの第５章に記載の方法により樹脂基板表面に水酸基、カルボキシル基またはアミノ基等を導入することができる。或いはこの方法により水酸基を導入した後に上記したガラス板の場合と同様にアミノ基やエポキシ基を有するシラン化合物を用いてアミノ基やエポキシ基を導入したり、更にはマレイミド基を導入することも可能である。ところで上記した固相への官能基の導入は、固相表面へのマトリクスの形成前に行なってもよく、或いはマトリクス形成後に行なってもよい。マトリクス形成前であれば固相表面にスピンコートやディップコート等の方法によって官能基の導入に必要な反応溶液を固相表面に供給すればよく、またマトリクス形成後であればインクジェット法等によって各ウェルに反応溶液を供給すればよい。

【００６９】また、樹脂基板にプローブを結合する方法としては例えば、特開昭６０－０１５５６０号公報に記載されている様に、樹脂基板表面を酸化処理して水酸基を導入し、次いでアミノ基を有するシラン化合物を含む

シランカップリング剤と該水酸基とを反応させてアミノ基を導入し、このアミノ基とプローブの官能基を反応させる方法が挙げられる。

【００７０】また、前処理後の基板が親水性の場合、他方の、マトリクスパターンを形成する相対的に撥水性の材料は、先に延べた樹脂製のマトリクス形成用の樹脂をそのまま用いることができる。また、さらなる撥水性が必要とされる場合にはマトリクス材料中に撥水剤を添加しておくこともできる。また、マトリクスパターンがフォトレジスト等の感光性樹脂で形成される場合には、露光、現像後にポストベークを適当な条件で行なうことによりマトリクスパターンにより強い撥水性を付与することも可能である。

【００７１】ここまでは、どちらかといえばプローブ溶液が親水性の場合について述べたが、プローブ溶液が親油的な場合には逆の処理をすればよいことになる。

【００７２】マトリクスパターンのウエルのサイズや形状は、基板のサイズ、最終的に作製されるアレイ全体のサイズ、アレイを構成するプローブ種類数、あるいは、マトリクスパターンの形成方法、マトリクスパターン間隙へのプローブ溶液等の供給方法、検出方法等によって適宜選択することができる。

【００７３】形状としては、図５に示す基板と平行な面の断面が正方形形状のものに加えて、長方形、各種多角形、円形、楕円形等種々の形状とすることができる。

【００７４】ウェルのサイズとしては、反応種の数、アレイ全体のサイズを考慮した場合、その最長幅が３００μｍ以下が望ましい。例えば、図５に示すように、ウエルの基板と平行な方向での断面を正方形とする場合にはその１辺の長さを２００μｍ以下とすることができる。更に、ウェルを長方形とする場合には、その長辺を２００μｍ以下、円形とする場合はその直径を２００μｍ以下とするのがより望ましい。その大きさの下限は例えば１μｍ程度とすることができる。

【００７５】各ウェルの配列形態は、図５のように平面図における上下方向で等間隔で配置する態様、隣り合う列でウェルの位置をずらして配置する態様等所望に応じて適宜変更可能である。

【００７６】隣接するウェル間の距離は、例えばインクジェット法でプローブ溶液をウェルに供給する際に吐出位置と供給されるべきウェルとの間に多少の位置ずれが生じてもそれがクロスコンタミネーションを生じさせないような間隔に設定することが好ましく、またアレイ全体のサイズ等とクロスコンタミネーションや、各種溶液の供給の際における操作性を考慮すると、隣接するウェル間の距離がウェルの最長幅の１／２～２倍の範囲にあることが好ましい。

【００７７】例えば、ウェルを正方形形状とする場合で、基板の大きさを、プローブ固定、試料供給、検出等の操作を自動化する場合に好適な大きさ（１インチ×１インチ、あるいは１ｃｍ×１ｃｍ）とすると、コンビナトリアルケミストリーとしての機能を十分に果たす必要性から１００個×１００個、あるいは、１０００個×１０００個以上のプローブ種類が存在することが望ましいので、マトリクス自体のサイズをも考慮して、ウェルの正方形形状の一辺を１～２００μｍ、隣接するウェル間の距離を２００μｍ以下とするのが望ましい。

【００７８】またマトリクスの厚さ（固相表面からの高さ）は、マトリクスパターンの形成方法やウェルの容量、供給するプローブ溶液の量等を考慮して決定されるが、好ましくは１～２０μｍとすることが好ましい。即ちこのような厚さとすることによって、例えばインクジェット吐出法を用いてプローブ溶液を各ウェルに供給する場合、インクジェット吐出条件との関連においてプローブ溶液の特性を、該プローブ溶液が該固相表面上に小さなスポットを形成することが困難な特性にしか調整し得ない場合であっても、該プローブ溶液を固相上の所定の位置に止めさせることができ、クロスコンタミネーションを極めて有効に防止することができる。

【００７９】仮に、上記の望ましい範囲の上限でのウェルの容積は、２００μｍ×２００μｍ×２０μｍ、すなわち、８００ｐｌとなる。また、このサイズで、隣接するウェル間の距離（図１のｘ）も同様に２００μｍとすると６２５個／ｃｍ^{2}のウェル密度が得られる。すなわちオーダーとして１０^{2}個／ｃｍ^{2}以上のウェル密度を有するアレイが得られる。また、ウエルを１辺が５μｍの正方形形状とし、隣接するウェル間の距離も５μｍとし、マトリクスパターンの厚さを４μｍとすると、ウェルの容積は０．１ｐｌとなり、その数は１００００００個／ｃｍ^{2}となる。５μｍ×５μｍ×４μｍのパターニングは現在の微細加工技術では現実的なサイズであるのでオーダーとして１０^{6}個／ｃｍ^{2}以上のウエル密度のアレイも本発明の発明の範囲となりうる。

【００８０】本態様においてプローブ溶液、あるいは、プローブと反応すべき物質のウェルへの供給液量は、例えばウェルの容積とほぼ同量とする場合には、上記の計算から、概ね０．１ピコリットル（ｐｌ）から1ナノリットル（ｎｌ）となる。また、マトリクスを供給される溶液に対して非親和性とした場合に、液種によっては、その表面張力によりウェルの容積を上回る量の液をウェルの開口上部に留めることが可能となる。そのような場合、例えば、ウェルの１０倍から数１０倍の液量を供給し、保持させることができる。すなわち、数ｐｌから数１０ｎｌの液を供給することになる。いずれの場合にも、このような少量の液のウェルへの供給は、一般的なマイクロディスペンサーやマイクロピペットでも可能であるが、位置精度と供給量精度を良好に供給することのできるインクジェット法を用いてプローブ溶液をウェルに供給することが好ましい。インクジェットプリントではμｍオーダーで高精度に位置決めをしてインクを吐出

するので、ウェルへの溶液の供給にはきわめて適しているといえる。また、吐出されるインクの量は一般的に数ｐｌから数１０ｎｌであるので、この点でもウェルへの溶液の供給に適しているといえる。

【００８１】本態様によれば、液滴の広がりは、核酸プローブと固相表面との反応とウェルにより定量的に制御され、また、吐出方向に多少の乱れがあっても、ウェルを含む領域に液滴が付着すれば、液滴のマトリクスにかかる部分は、マトリクスが吐出液に対して非親和性となっていることによって、その部分がはじかれ、ウェル内にスムーズに収納される。

【００８２】本発明に用いるインクジェット法は特に制限されないが、例えばピエゾジェット法、熱的な発泡を利用するバブルジェット法等が利用できる。

【００８３】ところで本発明において固相１０３として用いることのできる材料としては、固相表面に上記した様な種々の官能基を導入できるものであれば良く、更には第２の態様においては表面にマトリクスを形成できるものが好ましい。そしてプローブと標的物質との反応物の検出を光学的に行なう場合、固相を介した検出系を組む場合には固相を光学的に透明な固相とすることが好ましい。そのような材料としては例えば、合成石英、溶融石英等を含むガラス、シリコン、アクリル樹脂、ポリカーボネート樹脂、ポリスチレン樹脂、塩化ビニル樹脂等が挙げられる。また該反応物の光学的な検出を固相を介さないで検出する場合には光学的に黒色の固相を用いることが好ましく、カーボンブラック等の黒色の染顔料を含む樹脂基板等が用いられる。

【００８４】本発明ではこれらプローブアレイに反応すべき物質の溶液を供給し、適当な反応条件に置き、反応を行なう。個別のウェルに異なる反応すべき物質の溶液を供給する必要がある場合には、プローブアレイの複数のウェルのそれぞれに、プローブに反応させるべき少なくとも１種の物質が溶解した溶液を少なくとも１種供給する。この場合、上述のように、供給される溶液が、すでに形成されているプローブアレイのプローブが結合されているウェルに対して親和的であり、マトリクスパターンに非親和的であれば、供給領域を限定した、クロスコンタミネーションのない、定量的な液の供給が可能となる。表１に示した物質のように生体関連の物質の多くは水溶性なので、この場合にはウェルは親水性、マトリクスパターンは撥水性となる。また、これら反応すべき物質の供給にも、上述のように、インクジェット法を用いれば、微量な液量を、定量的に供給可能となる。

【００８５】本発明では、基板に結合するために供給するプローブの液量、または、反応すべき物質の液量が微量であるので、双方の反応条件が、供給された溶液の蒸発、気散を防ぐ条件を含んでいることが望ましい。

【００８６】以下実施例をもって本発明を更に詳細に説明する。

【００８７】実施例１
（バブルジェットプリンターを用いた核酸プローブアレイの製法、及びそのプローブアレイの評価）
（１）基板洗浄
１インチ角のガラス板をラックに入れ、超音波洗浄用洗剤に一晩浸した。その後、洗剤中で２０分間超音波洗浄を行い、その後、水洗により洗剤を除去した。蒸留水ですすいだ後、蒸留水の入った容器中でさらに超音波処理を２０分間行った。次に、予め８０℃に加温した１Ｎ水酸化ナトリウム溶液にガラス板を１０分間浸した。引き続き水洗、蒸留水洗浄を行って、プローブアレイ用のガラス板を用意した。

【００８８】（２）表面処理
アミノ基を結合したシラン化合物（Ｎ－β－（アミノエチル）－γ－アミノプロピルトリメトキシシラン）を含むシランカップリング剤（商品名：ＫＢＭ６０３；信越化学工業（株）社製）の１ｗｔ％水溶液を室温下で２時間撹拌し、上記シラン化合物の分子内のメトキシ基を加水分解した。次いでこの溶液に上記（１）で得た基板を室温（２５℃）で２０分間浸した後、引き上げて、窒素ガスをガラス板の両面に吹き付けて乾燥させた。次にガラス板を１２０℃に加熱したオーブン中で１時間ベークしてシランカップリング処理を完結させ、基板表面にアミノ基を導入した。次いでＮ－マレイミドカプロイロキシスクシンイミド（N-(6-Maleimidocaproyloxy)succinimide；Ｄｏｊｉｎ社製）（以降ＥＭＣＳと略）を２．７ｍｇ秤量し、ジメチルスルホキシド（ＤＭＳＯ）／エタノールの１：１溶液に最終濃度が０．３ｍｇ／ｍｌとなる様に溶解したＥＭＣＳ溶液を用意した。シランカップリング処理を行ったガラス板をこのＥＭＣＳ溶液に室温で２時間浸して、シランカップリング処理によってガラス板表面に担持されているアミノ基とＥＭＣＳ溶液のカルボキシル基を反応させた。この状態でガラス板表面にはＥＭＣＳ由来のマレイミド基が表面に存在することになる。ＥＭＣＳ溶液から引き上げたガラス板はＤＭＳＯ及びエタノールの混合溶媒及びエタノールで順次洗浄した後、窒素ガス雰囲気下で乾燥させた。

【００８９】（３）プローブＤＮＡの合成
ＤＮＡ自動合成機を用いて配列番号１の一本鎖核酸を合成した。なお配列番号１の一本鎖ＤＮＡ末端にはＤＮＡ自動合成機での合成時にチオールモディファイア（Ｔｈｉｏｌ－Ｍｏｄｉｆｉｅｒ）（グレンリサーチ（ＧｌｅｎＲｅｓｅａｒｃｈ）社製）を用いる事によってチオール（ＳＨ）基を導入した。続いて通常の脱保護を行いＤＮＡを回収し、高速液体クロマトグラフィーにて精製し、以下の実験に用いた。
配列番号：１
5' HS-$(CH_2)_6$-O-PO_2-O-ACTGGCCGTCGTTTTACA3'
（４）ＢＪプリンターによるＤＮＡ吐出、および基板への結合

上記配列番号１の一本鎖ＤＮＡを最終濃度が約４００ｍｇ／ｍｌになるようにＴＥ溶液（１０mM Ｔｒｉｓ－ＨＣｌ（ｐＨ８）／１mM ＥＤＴＡ水溶液）に溶解し、一本鎖ＤＮＡ溶液を調製した（正確な濃度は吸収強度から算出）。

【００９０】グリセリン７．５ｗｔ％、尿素７．５ｗｔ％、チオジグリコール７．５ｗｔ％、及び上記一般式（Ｉ）で示されるアセチレンアルコール（商品名：アセチレノールＥＨ；川研ファインケミカル（株）社製）１ｗｔ％を含む水溶液を用意し、上記ＤＮＡ溶液に加え、一本鎖ＤＮＡの最終濃度が８μＭとなるように調整した。この液体の表面張力は３０～５０ｄｙｎｅ／ｃｍの範囲内であり、また粘度は１．８ｃｐｓ（Ｅ型粘度計：東京計器（株）社製）であった。この液体をバブルジェットプリンター（商品名：ＢＪＣ６２０；キヤノン（株）社製）用インクタンクに充填しバブルジェットヘッドに装着した。なおここで用いたバブルジェットプリンター（商品名：ＢＪＣ６２０；キヤノン（株）社製）は平板への印刷が可能な様に改造を施したものである。またこのバブルジェットプリンターは３６０×７２０ｄｐｉの解像度で印字可能である。次いでこのプリンターに上記（２）で処理したガラス板を装着し、プローブ核酸を含む液体をガラス板上にスポッティングした。ここでバブルジェットヘッドの液体吐出面とガラス板の液体付着面との距離は１．２～１．５mmであった。またスポッティングは、３６０ｄｐｉの方向には１回のスポッティングの後２回の空吐出を行ない、７２０ｄｐｉの方向には１回のスポッティングの後５回の空吐出を行なう様に条件設定した。スポッティング終了後、ガラス板を３０分間加湿チャンバー内に静置し、ガラス板表面のマレイミド基と核酸プローブ末端のチオール基とを反応させた。なお上記プリンタの１吐出動作あたりのＤＮＡプローブ溶液の吐出量は約２４ｐｌであった。

【００９１】（５）ブロッキング反応

マレイミド基とチオール基との反応終了後、ガラス板を１Ｍ ＮａＣｌ／５０mMリン酸緩衝液（ｐＨ７．０）溶液で洗浄し、ガラス板表面のＤＮＡを含む液体を完全に洗い流した。次いでガラス板を２％ウシ血清アルブミン水溶液中に浸して２時間放置し、ブロッキング反応を行った。

【００９２】（６）ハイブリダイゼーション反応

配列番号１のＤＮＡと相補的な塩基配列を有する一本鎖ＤＮＡをＤＮＡ自動合成機で合成し、５’末端にローダミンを結合させて標識化した一本鎖ＤＮＡを得た。この標識化一本鎖ＤＮＡを１Ｍ ＮａＣｌ／５０mMリン酸緩衝液（ｐＨ７．０）に最終濃度１μＭとなるように溶解し、この溶液中に上記（５）で得たブロッキング処理したプローブアレイを浸漬し、室温（２５℃）で３時間ハイブリダイゼーション反応を行った。その後、プローブアレイを１Ｍ ＮａＣｌ／５０mMリン酸緩衝液（ｐＨ７．０）溶液で洗浄してプローブ核酸とハイブリダイズしなかった一本鎖ＤＮＡを洗い流した。次に該プローブアレイのスポットの蛍光量を、画像解析装置（商品名：ＡＲＧＵＳ ５０；浜松ホトニクス（株）社製）を接続し、ローダミンＢに適するフィルターセットを装着した倒立型蛍光顕微鏡を用いて定量した。

【００９３】（７）結果

標識化一本鎖ＤＮＡと完全マッチである配列番号１の核酸プローブのスポットでは４６００の蛍光量であった。またハイブリダイゼーション後の、各スポットが蛍光発光している状態のプローブアレイを蛍光顕微鏡（ニコン（株）社製）を用いて観察した。その結果本実施例にかかるプローブアレイでは、

ａ）各々のスポットがほぼ円形であって、またその直径が約７０～１００μｍの範囲内にあること、

ｂ）隣接するスポットとの間には各々のスポットの直径と略等しい、約１００μｍのスペースが有り、各々のスポットが互いに明確に独立していること、

ｃ）スポットの行と列が揃っていることが明らかとなった。

【００９４】このことはプローブアレイ上でハイブリダイズしたスポットの自動検出等を行わせる上で極めて有効である。

【００９５】実施例２

（バブルジェットプリンタを用いた核酸プローブアレイの製造、及びそのプローブアレイを用いた標的核酸の検出）

（１）上記実施例１の（１）及び（２）と全く同様にしてプローブアレイ用の表面処理を施したガラス板を用意した。

【００９６】（２）プローブＤＮＡの合成

ＤＮＡ自動合成機を用いて配列番号１～４の一本鎖核酸を合成した。なお配列番号１～４の一本鎖核酸は、実施例１で用いた配列番号１を基本とし、１塩基変化させたものを配列番号２、３塩基変化させたものを配列番号３、そして６塩基変化させたものを配列番号４とした。また配列番号１～４の一本鎖ＤＮＡ末端にはＤＮＡ自動合成機での合成時にＴｈｉｏｌ－Ｍｏｄｉｆｉｅｒ（ＧｌｅｎＲｅｓｅａｒｃｈ社製）を用いる事によってチオール（ＳＨ）基を導入した。続いて通常の脱保護を行いＤＮＡを回収し、高速液体クロマトグラフィーにて精製し、以下の実験に用いた。配列番号２～４の配列を以下に示す。

配列番号：２

5' HS-$(CH_2)_6$-O-PO_2-O-ACTGGCCGTTGTTTTACA 3'

配列番号：3

5' HS-$(CH_2)_6$-O-PO_2-O-ACTGGCCGCTTTTTTACA 3'

配列番号：4

5' HS-$(CH_2)_6$-O-PO_2-O-ACTGGCATCTTGTTTACA 3'

（３）ＢＪプリンターによるＤＮＡプローブの吐出、お

よび基板への結合

上記配列番号１～４の一本鎖ＤＮＡを用いて、上記実施例１の（４）に記載した方法と同様の方法で４種類の吐出用液体を調製し、実施例１で用いたバブルジェットプリンタ用の４つのインクタンクに各々の液体を充填し、各々のインクタンクをバブルジェットヘッドに装着した。次いで該プリンタに上記（１）と同じ方法で作成したガラス板を装着し、該ガラス板上に４種の核酸プローブの各々を該ガラス板の３×３ｍｍの４つのエリアの各々にスポッティングした。なお各エリア内でのスポッティングのパターンは実施例１と同様とした。スポッティング終了後、ガラス板を３０分間加湿チャンバー内に静置し、マレイミド基とチオール基とを反応させた。

【００９７】（４）ブロッキング反応

マレイミド基とチオール基との反応終了後、ガラス板を１Ｍ　ＮａＣｌ／５０ｍＭリン酸緩衝液（ｐＨ７．０）溶液で洗浄し、ガラス板表面のＤＮＡ溶液を完全に洗い流した。次いでガラス板を２％ウシ血清アルブミン水溶液中に浸して２時間放置し、ブロッキング反応を行った。

【００９８】（５）ハイブリダイゼーション反応

配列番号１のＤＮＡと相補的な塩基配列を有する一本鎖ＤＮＡをＤＮＡ自動合成機で合成し、５’末端にローダミンを結合させて標識化一本鎖ＤＮＡを得た。この標識化一本鎖ＤＮＡを１Ｍ　ＮａＣｌ／５０ｍＭリン酸緩衝液（ｐＨ７．０）に最終濃度１μＭとなるように溶解し、（４）で得られたプローブアレイとハイブリダイゼーション反応を３時間行った。その後、プローブアレイを１Ｍ　ＮａＣｌ／５０ｍＭリン酸緩衝液（ｐＨ７．０）溶液にて洗浄してプローブ核酸とハイブリダイズしなかった一本鎖ＤＮＡを洗い流した。次に該プローブアレイの各々のスポットを蛍光顕微鏡（ニコン（株）社製）で観察し、その蛍光量を、画像解析装置（商品名：ＡＲＧＵＳ　５０；浜松ホトニクス（株）社製）を接続し、ローダミンＢに適するフィルターセットを装着した倒立型蛍光顕微鏡を用いて定量した。

【００９９】（６）結果

標識化一本鎖ＤＮＡと完全マッチである配列番号１のＤＮＡプローブのスポットでは４６００の蛍光量であるのに対し、１塩基のミスマッチ配列を有する配列番号２のＤＮＡプローブのスポットでは、２８００の蛍光量が得られた。また、３塩基ミスマッチを有する配列番号３のＤＮＡプローブのスポットでは、２１００と完全マッチの半分以下の蛍光量しか得られず、６塩基ミスマッチの配列番号４のＤＮＡでは蛍光は観測されなかった。以上の事から、ＤＮＡアレイ基板上で完全相補性の一本鎖ＤＮＡを特異的に検出することができた。

【０１００】実施例３

（液体中のＤＮＡプローブの濃度とバブルジェット吐出特性）

（１）ＤＮＡプローブの合成

以下に示す配列番号５の配列を有する一本鎖ＤＮＡをＤＮＡ自動合成機を用いて合成し、それを濃度が各々約０．２ｍｇ／ｍｌ、２ｍｇ／ｍｌ、１５ｍｇ／ｍｌになるようにＴＥ溶液（１０ｍＭ　Ｔｒｉｓ－ＨＣｌ（ｐＨ８）／１ｍＭ　ＥＤＴＡ水溶液）に溶解し、濃度の異なる３種類のＤＮＡプローブ溶液を調製した（正確な濃度は吸収強度から算出した）。

配列番号：５

5' GCCTGATCAGGC 3'

（２）ＢＪプリンターによる吐出

グリセリン７．５％、尿素７．５％、チオジグリコール７．５％、上記一般式（Ｉ）で示される構造を有するアセチレンアルコール（商品名：アセチレノールＥＨ；川研ファインケミカル（株）社製）１％を含む水溶液を用意し、この水溶液を上記（１）で調整した濃度０．２ｍｇ／ｍｌのプローブ溶液に加えて、最終濃度が約０．０２ｍｇ／ｍｌ（３μＭ）に希釈した。この液体を上記実施例１で用いたバブルジェットプリンタ用のインクタンクに充填し、このインクタンクを実施例１で用いたバブルジェットプリンタのヘッドに装着した。

【０１０１】次に該プリンタにＡ４サイズのアルミ板を装着し、該アルミ板の３×５平方インチのエリアに対してスポッティングを行った。ここでのスポッティングは、上記エリアに３６０×７２０ｄｐｉの密度でスポッティングされるように設定した。また最初にコントロールとしてＢＪ６２０用の市販のインクを該アルミ板上に印字した。この操作を計４枚のアルミ板に対して行った。

【０１０２】次に各々のアルミ板上にスポットされた核酸プローブをＴＥ溶液を用いて回収し、ゲル濾過法により精製し、精製された回収核酸プローブの量を吸収スペクトルにより測定した。ここで理論的に求められる核酸プローブの回収量は以下の通りである。即ち本実施例に用いたプリンターのヘッドから吐出される液滴１つあたりの体積は２４ピコリットルである。そして３６０×７２０ｄｐｉの密度で３×５平方インチのエリアにスポッティングしたアルミ板が４枚であるから、

２４（ピコリットル）×（７２０×３６０）×（３×５）×４枚＝３７３μｌ

となる。この量のプローブ核酸が示す２６０ｎｍにおける吸光度と回収された核酸プローブの２６０ｎｍにおける吸光度を図３に示す。

【０１０３】上記（２）と全く同様の操作を、濃度２ｍｇ／ｍｌ、１５ｍｇ／ｍｌ各々のプローブ溶液について行った。なお各々の吐出用液体の核酸プローブの最終濃度は３０μＭ（０．２ｍｇ／ｍｌ）及び２２５μＭ（１．５ｍｇ／ｍｌ）とした。各溶液から回収されたプローブ核酸が示す吸光度及び理論的に求められたプローブ核酸量が示す吸光度の結果を図３に示す。

【０１０４】（３）結果

図３から分かるように核酸プローブの実際の吐出量は理論的に予想される値に近い値であった。この事からバブルジェット法を用いての核酸プローブの吐出において、バブルジェットヘッドのヒータ部への核酸プローブの焦げ付きなどによる核酸プローブの量的損失は認められない。また各々の濃度の液体を用いてのアルミ板へのスポッティング工程中、ヘッドのトラブル、例えば不吐出等は一切発生しなかった。またコントロールとしてアルミ板にスポッティングしたバブルジェットプリンター用インクのスポットと核酸プローブのスポットを目視にて対比したところ、濃度３μＭ及び３０μＭの液体を用いて作成したスポットのスポッティング状況は、インクスポットのそれと殆ど同様であった。また濃度２２５μＭの液体を用いて作成したスポットはインクスポットと比較して若干の乱れが認められた。

【０１０５】実施例４

（バブルジェットプロセスが核酸プローブに与える影響の検討）

（１）核酸プローブの合成

アデニン（以降「Ａ」と記載）からなる塩基長１０ｍｅｒ（合成品）、ｏｌｉｇｏＡ（４０−６０ｍｅｒ；ファルマシア社製）、ｐｏｌｙ（ｄＡ）（３００〜４００ｍｅｒ；ファルマシア社製）をそれぞれＴＥ溶液で希釈して最終濃度が１ｍｇ／ｍｌになるよう調製し、長さの異なる核酸プローブ溶液を用意した。なお１０ｍｅｒの塩基配列（配列番号：６）は以下の通りである。

配列番号：６

5' AAAAAAAAAA 3'

（２）バブルジェットプリンターによるＤＮＡ溶液の吐出

グリセリン７．５ｗｔ％、尿素７．５ｗｔ％及び上記一般式（Ｉ）で示されるアセチレンアルコール（商品名：アセチレノールＥＨ；川研ファインケミカル）１ｗｔ％を含む水溶液を用意し、この水溶液で上記（１）で作成した各々の核酸プローブ溶液を最終濃度が約０．１ｍｇ／ｍｌとなるように希釈した。

【０１０６】実施例３と同様カートリッジに充填した各々の核酸プローブ溶液をアルミ板上に吐出させ、スポッティング状況を目視にて観察した。その結果塩基長１０ｍｅｒ及び４０〜６０ｍｅｒの核酸プローブに関しては、アルミ板上に独立したスポットが整然と並んだプローブアレイが得られた。また３００〜４００ｍｅｒの核酸プローブに関しても、基本的には同様のプローブアレイが得られたが、隣接するスポット同士が繋がっている部分が認められた。これは核酸プローブの塩基鎖が長い＊ことに起因する液体の物性変化が生じ、バブルジェットヘッドからの吐出の方向性が若干不正確になった為と考えられる。

【０１０７】次に各々の核酸プローブ溶液を用いて作成したプローブアレイ上のスポットを実施例３と同様にして回収した。回収した核酸プローブ溶液１００μｌを逆相ＨＰＬＣで分析し、吐出前の溶液との比較によって核酸プローブの切断の有無を調べた。なお逆相ＨＰＬＣの溶出は１Ｍトリエチルアミンアセテートを含む７〜７０％アセトニトリル濃度勾配により行った。その結果、切断されたと考えられる様なＤＮＡ断片は観測されず、よって核酸プローブはバブルジェット法での吐出によっても変質を受けなかったことが確認できた。また回収した核酸プローブの定量を実施例３と同様にして行った結果、図４に示すように３種類の長さの核酸プローブはほぼ理論値通りの量が回収された。

【０１０８】実施例５

（反応時間の検討）実施例１の（４）において、核酸プローブがスポッティングされた表面処理ガラス板を加湿チャンバー中に１０分、９０分、一晩室温（２５℃）放置した以外は実施例１と同様にしてプローブアレイを製造し、各々のプローブアレイをハイブリダイゼーション反応に供した。その結果９０分、及び一晩反応させたプローブアレイについては、全て実施例１で得られたプローブアレイが示す蛍光強度と同程度の蛍光強度を与えた。このことからガラス板表面のマレイミド基と核酸プローブ末端のチオール基との結合反応は３０分でほぼ終了している事が明らかになった。一方反応時間が１０分のプローブアレイは実施例１のそれに比べて、７０％程度の蛍光量であった。

【０１０９】実施例６

（バブルジェットプリンタを用いたＰＮＡプローブアレイの製造、及びそのプローブアレイを用いた標的核酸の検出）

（１）上記実施例１の（１）及び（２）と全く同様にしてプローブアレイ用の表面処理を施したガラス板を用意した。

【０１１０】（２）プローブＰＮＡの合成

下記配列番号７及び８の塩基配列を有するプロテイン核酸（ＰＮＡ）（日本パーセプティブ（株）社製）を用意した。このＰＮＡはＮ末端（ＤＮＡの５’末端に相当）にシステイン残基（Ｃｙｓと表記）が結合され、その結果としてＮ末端にチオール基が導入されている。また配列番号８のＰＮＡプローブは配列番号７のＰＮＡプローブを一塩基変化させたものである。

配列番号：７

NCys-NH$(CH_2)_2$-O-$(CH_2)_2$-O-CH_2CONH-ACTGGCCGTCGTTTTACA^C

配列番号：８

NCys-NH$(CH_2)_2$-O-$(CH_2)_2$-O-CH_2CONH-A59TGGCCGTTGTTTTACA^C

（3）ＢＪプリンターによるＰＮＡプローブの吐出、および基板への結合

上記各々のＰＮＡプローブを１００μｌの０．１ｗｔ％トリフルオロ酢酸に最終濃度が８０μＭとなる様に溶解し、次いでグリセリン７．５ｗｔ％、尿素７．５ｗｔ％、チオジグリコール７．５ｗｔ％、及び上記一般式（Ｉ）で示されるアセチレンアルコール（商品名：アセチレノールＥＨ；川研ファインケミカル（株）社製）１ｗｔ％を含む水溶液を上記ＰＮＡのトリフルオロ酢酸溶液に加えて、ＰＮＡプローブの最終濃度が８μＭとなるように調整した。この液体の表面張力は３０～５０ｄｙｎ／ｃｍの範囲内であり、また粘度は１～５ｃｐｓの範囲内であった。

【０１１１】このＰＮＡプローブ溶液各々を、実施例２の（３）に記載したのと同様にして上記（１）で作成したガラス板上の各々のエリアにスポッティングした。スポッティング終了後、３時間加湿チャンバー内に静置し、マレイミド基とチオール基とを反応させた。

【０１１２】なお上記プリンタの１吐出動作あたりのＰＮＡプローブ溶液の吐出量は約２４ｐｌであった。

【０１１３】（4）ブロッキング反応

マレイミド基とチオール基との反応終了後、ガラス板を１Ｍ ＮａＣｌ／５０mMリン酸緩衝液（ｐＨ７．０）溶液で洗浄し、ガラス板表面のＰＮＡを含む液体を完全に洗い流した。次いでガラス板を２％ウシ血清アルブミン水溶液中に浸して３時間放置し、ブロッキング反応を行った。

【０１１４】（5）ハイブリダイゼーション反応

配列番号７のＰＮＡと相補的な塩基配列を有する一本鎖ＤＮＡをＤＮＡ自動合成機で合成し、５'末端にローダミンを結合させて標識化した一本鎖ＤＮＡを得た。この標識化一本鎖ＤＮＡを１０mMリン酸緩衝液（ｐＨ７．０）に最終濃度５ｎＭとなるように溶解し（溶液量１ｍｌ）、このＤＮＡ溶液中に上記（４）で得たブロッキング処理したＰＮＡプローブアレイを浸漬し、室温（２５℃）で１２時間ハイブリダイゼーション反応を行った。その後、プローブアレイを１０mMリン酸緩衝液（ｐＨ７．０）溶液で洗浄してＰＮＡプローブとハイブリダイズしなかった一本鎖ＤＮＡを洗い流した。次に該プローブアレイのスポットの蛍光量を、画像解析装置（商品名：ＡＲＧＵＳ ５０；浜松ホトニクス（株）社製）を接続し、ローダミンＢに適するフィルターセットを装着した倒立型蛍光顕微鏡を用いて定量した。

【０１１５】（6）結果

標識化一本鎖ＤＮＡと完全マッチである配列番号７のＰＮＡプローブでは２４００の蛍光量であったのに対して１塩基ミスマッチ配列を有する配列番号８のＰＮＡプローブでは約半分の１１００であった。以上のことからＰＮＡアレイ上で完全相補性の一本鎖ＤＮＡを特異的に検出することができた。

【０１１６】またハイブリダイゼーション後の、各スポットが蛍光発光している状態のプローブアレイを蛍光顕微鏡（ニコン（株）社製）を用いて観察した。その結果本実施例にかかるプローブアレイでは、

ａ）各々のスポットがほぼ円形であって、またその直径が約２００μｍの範囲内にあること、

ｂ）隣接するスポットとの間には、約５０μｍのスペースが有り、各々のスポットが互いに明確に独立していること、

ｃ）スポットの行と列が揃っていることが明らかとなった。

【０１１７】このことはプローブアレイ上でハイブリダイズしたスポットの自動検出等を行わせる上で極めて有効である。

【０１１８】更にハイブリダイゼーション反応時、及びその後の未反応の一本鎖ＤＮＡの除去に用いる溶液に塩化ナトリウムを含有させる必要が無いため、蛍光の観察中に塩化ナトリウムの析出に注意する必要がなく、プローブアレイ上のハイブリッドの検出をより容易に行うことができた。また保存上も密封の必要がなく、取扱いが容易であった。

【０１１９】なおＰＮＡプローブのスポット径が実施例１で得たプローブアレイのスポットよりも大きい理由は明らかでないが、本発明者らはＰＮＡプローブはＤＮＡプローブと比較して若干水溶性が劣るとの知見を得ており、両者の水溶性の差が各々のインクジェット吐出液の表面張力に差異を生じさせる結果、スポット径が異なっているものと推測される。

【０１２０】実施例７

（表面にエポキシ基を導入したプローブアレイ用ブラックマトリクス付ガラス基板の調製及びその評価）

（１）合成石英からなるガラス基板（５０mm×５０mm）を、２ｗｔ％水酸化ナトリウム水溶液を用いて超音波洗浄し、次いでＵＶオゾン処理を行なって表面を清浄化した。エポキシ基を結合したシラン化合物（γ－グリシドキシプロピルトリメトキシシラン）を含むシランカップリング剤（商品名：ＫＢＭ４０３；信越化学工業株式会社製）を１ｗｔ％含有する５０ｗｔ％メタノール水溶液を室温下で３時間攪拌し、上記シラン化合物中のメトキシ基を加水分解した。ついでこの溶液を上記基板表面にスピンコーターで塗布し、１００℃で５分間加熱、乾燥して基板表面にエポキシ基を導入した.

（２）次にカーボンブラックを含有するＤＥＥＰ－ＵＶレジスト（ブラックマトリクス用ネガ型レジスト）（商品名：ＢＫ－７３９Ｐ；新日鉄化学株式会社製）をスピンコータで硬化後の膜厚が５μｍとなるように塗布し、この基板をホットプレートで８０℃で５分間加熱して硬化させた。ＤＥＥＰ－ＵＶ露光装置を用いて１ｃｍ×１ｃｍの領域に、図５における隣接ウェル間の距離（Ｘ）が１００μｍ、及びウェルの形状が１００μｍ×１００

μmの正方形となるようにパターニングされたマスクを用いてプロキシミティ露光し、次いで無機アルカリ水溶液の現像液で、スピン現像機を用いて現像し、更に純水で洗浄して現像液を完全に除去した。次にスピン乾燥機を用いて簡単に乾燥し、その後クリーンオーブン中で１８０℃で３０分間加熱してレジストを本硬化させ、所定の配列でウェルが２５００個配置され、隣接するウェルがブラックマトリクスで隔離された基板を得た。なお各ウェルの容積は５０ピコリットル（ｐｌ）と計算される。この時点でブラックマトリクス表面の水に対する接触角は９３°と濡れにくく、またウェル底面の水に対する接触角は３５°と濡れやすかった。

【０１２１】（３）１０μＭのローダミンＢ水溶液をバブルジェットプリンター（商品名：ＢＪＣ６２０：キヤノン（株）社製）用インクタンクに充填し、前記実施例１で用いたバブルジェットプリンタのバブルジェットヘッドに装着した。そして上記（１）及び（２）で用意した固相をプリンタに装着し、該固相のウェルに、市松パターン（ひとつおき）にローダミンＢ水溶液を供給した。なお１ウェルあたりの供給量は約５０ｐｌである。またこのプリンタの吐出位置決め精度は±２．５μｍである。次に１０μＭのアミノＦＩＴＣの水溶液を別のインクタンクに充填し、上記プリンタのバブルジェットヘッドに装着して、先にローダミンＢ水溶液を供給したウェルに隣接する別のウェルに供給した。ここでローダミンＢ及びアミノＦＩＴＣを用いたのは水溶性でありインクジェットヘッドからの吐出が容易に行なえること、及び蛍光の観察によってウェルに供給された液体の状態やクロスコンタミネーションを確認できる為である。

【０１２２】（４）蛍光顕微鏡（ニコン（株）社製）にＧ励起フィルター（ローダミンＢ用）、Ｂ励起フィルター（アミノＦＩＴＣ用）を装着し、倍率１００倍にてウェルに供給された各々の水溶液の状態を蛍光で観察した。その結果各々の水溶液とも、液滴を形成することなくウェル内に均一に供給されていた。また各々のウェルからは互いに他の色素の蛍光は観察されず、クロスコンタミネーションは認められなかった。

【０１２３】実施例８

（実施例７の基板を用いたプローブアレイの調製及びそれを用いた標的核酸の検出）

（１）実施例７と同様の方法によりブラックマトリクス（ＢＭ）付の基板を作成した。

（２）ＤＮＡプローブとして５’末端の水酸基にリン酸基とヘキサメチレンを介してアミノ基を結合した１８量体のオリゴマー（配列番号：９）、配列番号９のオリゴマーに対して１個のヌクレオチドがミスマッチのプローブ（配列番号：１０）、及び配列番号９のオリゴマーに対して２個のヌクレオチドがミスマッチのプローブ（配列番号：１１）（全て日本製粉株式会社製、ＨＰＬＣグレード）を用意した。配列番号９のオリゴマーの塩基配列は、一本鎖ＤＮＡであるＭ１３ｍｐ１８－ｓｓＤＮＡのマルチプルクローニングサイトの一部の塩基配列に相補的な配列である。以下に配列番号：９～１１の塩基配列とリンケージの構造を示す。

配列番号：９

$5'NH_2-(CH_2)_6-O-PO_2-O-\text{TGTAAAACGACGGCCAGT}^{3'}$

配列番号：１０

$5'NH_2-(CH_2)_6-O-PO_2-O-\text{TGTAAAAC}\underline{\text{C}}\text{ACGGCCAGT}^{3'}$

配列番号：１１

$5'NH_2-(CH_2)_6-O-PO_2-O-\text{TGTA}\underline{\text{T}}\text{AAC}\underline{\text{C}}\text{ACGCCCAGT}^{3'}$

（３）上記配列番号９～１１のＤＮＡプローブに対して完全相補的な一本鎖ＤＮＡを合成した。次にＮａＣｌを５０mMの濃度で含むＴＥ溶液（ｐＨ８）に、各ＤＮＡプローブ及び一本鎖ＤＮＡを最終濃度が１００μＭとなるように溶解し、ＤＮＡプローブ溶液及び一本鎖ＤＮＡ溶液を調製した。そしてＤＮＡプローブを含む溶液１００μｌに対して各々のＤＮＡプローブに相補的な一本鎖ＤＮＡを含む溶液を１００μｌ加えて混合し、各々の混合溶液を９０℃から２５℃まで直線的に２時間かけて冷却し、各々のＤＮＡプローブと各々の一本鎖核酸とのハイブリッドを形成させた。次に上記配列番号：９～１１の各ＤＮＡプローブのハイブリッドを含む溶液を、グリセリン７．５ｗｔ％、尿素７．５ｗｔ％、チオジグリコール７．５ｗｔ％、及び前記一般式（Ｉ）で示されるアセチレンアルコール（商品名：アセチレノールＥＨ；川研ファインケミカル（株）社製）１ｗｔ％を含む水溶液に加え、ハイブリッドの最終濃度が８μＭとなるように調整した。各々のＤＮＡプローブのハイブリッドを含むこれらの液体の表面張力は何れも３０～５０ｄｙｎｅ／ｃｍの範囲内であり、また粘度も１～５ｃｐｓ（Ｅ型粘度計：東京計器（株）社製）の範囲内であった。

【０１２４】次にバブルジェットプリンター（商品名：ＢＪＣ６２０；キヤノン（株）社製）用インクタンクを３個用意し、各々のインクタンクに上記の３種のハイブリッド溶液を充填し、実施例１で用いたバブルジェットプリンタのヘッドに装着した。また上記（１）及び（２）で作成したＢＭ付ガラス基板をセットし、まず配列番号９のＤＮＡプローブのハイブリッドを含む溶液を１列目のウェル（図６の１３１）に供給した。次に配列番号１０のＤＮＡプローブのハイブリッドを含む溶液を上記１列目のウェルに隣接する２列日目ウェル（図６の１３３）に供給し、更に配列番号１１のＤＮＡプローブのハイブリッドを含む溶液を上記２列目のウェルに隣接する３列目のウェル（図６の１３５）に供給した。なお１つのウェルに対して何れのハイブリッド溶液を４回吐出して約１００ｐｌ供給した。この量は１つのウェルの容積の約２倍であるが、顕微鏡で各ウェルを観察したところ、供給されたハイブリッド溶液はウェルの開口部からは盛り上がって存在しているが、疎水性のマトリクスによってウェル内に止まっており、ウェル間でのクロス

コンタミネーションは観察されなかった。

【０１２５】次に基板を２５℃、湿度１００％の恒温恒湿槽に１２時間置き、プローブのアミノ基とウェルのエポキシ基とを反応させた。なおプローブの塩基のアミノ基は完全相補的な一本鎖ＤＮＡとハイブリッドを形成しているため、各ウェルのエポキシ基と反応することはない。

【０１２６】（４）次に基板を８０℃の純水で１０分間洗浄し、基板に結合しているプローブとハイブリッドを組んでいる相補鎖をプローブから解離させると共に洗い流した。次いで基板を１％エタノールアミン水溶液で室温下で１時間処理し、各ウェル内の未反応のエポキシ基を開環させた。次に基板を純水で洗浄、乾燥した。

【０１２７】上記（４）の操作によってウェル内のＤＮＡプローブと反応しなかったエポキシ基は開環して水酸基となり、また反応させたエタノールアミンにも水酸基が存在するためウェルの底面はより親水性が高くなり、後述の標的一本鎖ＤＮＡを含む溶液のウェルへの供給の際に有利となる。

【０１２８】（５）次にＮａＣｌを５０mMの濃度で含むＴＥ溶液（ｐＨ８）に配列番号９のＤＮＡプローブに対する完全相補性の一本鎖ＤＮＡを最終濃度が１０μＭとなるように溶解し、この溶液に上記（４）で得たウェルにエポキシ基を導入したプローブアレイを浸漬し、８０℃から２５℃まで２時間かけて降温しハイブリタイゼーション反応を行なった。ついで２０℃で１０mMのＮａＣｌを含むＴＥ緩衝液（ｐＨ８）で基板を２０分間洗浄したのちスピン転燥機で表面の洗浄液を除去した。

【０１２９】（６）次にＮａＣｌを５０mMの濃度で含むＴＥ溶液（ｐＨ８．０）に、二本鎖核酸にインターカレートして初めて蛍光を発する、２－メチル－４、６－ビス（４－Ｎ，Ｎ－ジメチルアミノフェニル）ピリリウムアイオタイド（以下「Ｐ２」と略）をその濃度が１０μＭとなるように溶解し、この溶液を上記インクジェットプリンタ用のインクタンクに充填して上記インクジェットプリンタのヘッドに取り付けた。また上記（５）にてハイブリダイゼーションを行なった基板を上記プリンタにセットし、各々のウェルに対してＰ２溶液を１００ｐｌづつ供給したのち、乾燥を防止するために湿度１００％の専用チャンバー内で５分間放置し、チャンバー内に保持したまま倒立型の顕微鏡（商品名：ＩＭＴ２；オリンパス光学株式会社製、倍率：１００倍、蛍光顕微鏡用のフィルターキューブ（励起用フィルター４５５ｎｍから５９５ｎｍ（透過）、ダイクロイックミラー６２０ｎｍ、蛍光用バリアーフィルター６１０ｎｍから７２５ｎｍ（透過））を使用）にＩＣＣＤカメラ（商品名：Ｃ２４００－８７；浜松ホトニクス社製）とイメージプロセッサ（商品名：ＡＲＧＵＳ　５０；浜松ホトニクス社製）を接続し、蛍光を観察定量した。なお観察エリアは２５μｍ×２５μｍ、インテグレーション×６４、ＡＲＧＵＳ　５０の増幅レベルは適宜設定した。

【０１３０】その結果、配列番号１１のＤＮＡプローブを結合させたウェルからは、バックグラウンドとほぼ同様の、１２００～１５００の蛍光強度が観察された。一方配列番号９のＤＮＡプローブを結合させたウェルからは、９８００～１０３００の蛍光強度が観察され、また配列番号１０のＤＮＡプローブを結合させたウェルからは、３５００～３９００の蛍光強度が観察された。更に各固相をＴＥ緩衝液を用いて３５℃で１０分間洗浄して再度蛍光強度を測定したところ、配列番号１０のＤＮＡプローブを結合させたウェルからはバックグラウンドと同程度の蛍光強度しか観察されなくなった。

【０１３１】これらの結果から、本実施例に係るプローブアレイを用いることで、各ウェルにおいてハイブリダイゼーション反応を行なうことができ、更に配列番号９と完全相補的な標的核酸を特異的に検出できることが分かった。

【０１３２】実施例９

（実施例８のプローブアレイの各ウェルへの反応物質の選択的供給及びプローブとの反応）

（１）実施例８と同様にして配列番号９～１１のＤＮＡプローブを結合させた基板を用意した。

【０１３３】（２）配列番号９～１１のＤＮＡプローブに対して完全相補的な３種類の一本鎖ＤＮＡを合成した。ＮａＣｌを５０mMの濃度で含むＴＥ溶液（ｐＨ８）に上記３種類の一本鎖ＤＮＡを各々の濃度が１００μＭとなるように溶解した。バブルジェットプリンター（商品名：ＢＪＣ６２０；キヤノン（株）社製）用インクタンクを３個用意し、各々のインクタンクに上記の３種の一本鎖ＤＮＡ溶液を充填し、実施例１で用いたバブルジェットプリンタのヘッドに装着した。また上記（１）で用意した基板もプリンタにセットし、配列番号９～１１のＤＮＡプローブが結合しているウェルに対して各々完全相補的な一本鎖ＤＮＡを含む溶液を１つのウェルにつき１００ｐｌづつ供給した。この時点で各ウェルの状態を顕微鏡で観察したところ、液のにじみ、クロスコンタミネーションは観察されず、またプローブアレイの各ウェルに個別に反応させるべき物質の溶液を供給できることが分かった。

【０１３４】（３）次に実施例８と同様にして各ウェルにおいてハイブリダイゼーション反応を行なわせた後、実施例８と同様にしてＰ２溶液を各ウェルに供給し、蛍光を観察することでハイブリッドの検出を行なった。その結果、全てのウェルから９８００～１０３００の強度の蛍光が観察された。このことから固相プローブアレイの各ウェルに個別に反応物質を供給し、各ウェルにおいてプローブと反応物質を反応させ、そして反応の結果物を検出できることが確認された。

【０１３５】実施例１０

（実施例７の基板のウェル底面の親水化処理）

（１）実施例７と同様にしてブラックマトリックスパターンを有するガラス基板を用意した。

【０１３６】（２）この基板のブラックマトリックスが形成されている側の表面にＵＶオゾン処理を行なった。この時点でブラックマトリックス表面の水に対する接触角は９３°と濡れにくい状態であり、ウェル底面の水に対する接触角は２２°であって実施例７で得たブラックマトリクス付基板のウェル底面のそれと比較して濡れやすい状態であった。これは上記ＵＶオゾン処理による効果と考えられる。

【０１３７】（３）次に実施例７と同様にしてローダミンＢ、及びアミノＦＩＴＣの水溶液を用いてウェルへのインクジェット吐出液の供給状況を観察したところ、各々の水溶液は共にウェル内で液滴を形成することなくウェル内に均一に供給されていた。プローブアレイの固相として表面にウェルを備えた固相を用いる場合には、表面にウェルを有しない、平坦且つ均一な表面特性を有する固相を用いる場合と異なり、インクジェット吐出液を出来るだけ限定された位置に留めなくてもよく、むしろウェル底面に十分にインクジェット吐出液を行き亘らせることが、後に行なうプローブと標的物質との反応の検出にはより有利となる。本実施例に記載したウェル底面の親水化処理はその一実施態様として好ましい方法である。また各々の色素が供給されるウェルからは互いに他の色素は観察されず、クロスコンタミネーションを生じさせることなしに、各々のウェルに各々の色素水溶液をインクジェットプロセスを用いて供給できたことが分かった。

【０１３８】実施例１１

（ＢＭ形成基板の各ウェルにプローブ固定用官能基導入の為の液体をインクジェット法にて供給して得た固相を用いたプローブアレイの製法及びその使用）

（１）実施例７と同様にしてブラックマトリックスを備えた基板を用意した。

【０１３９】（２）アミノ基を結合したシラン化合物（Ｎ－β－（アミノエチル）－γ－アミノプロピルトリメトキシシラン）を含むシランカップリング剤（商品名：ＫＢＭ６０３；信越化学工業株式会社製）を１ｗｔ％含有する１０ｗｔ％メタノール水溶液を室温下で３時間攪拌し、上記シラン化合物中のメトキシ基を加水分解した。ついでこの溶液をバブルジェットプリンター（商品名：ＢＪＣ６２０；キヤノン（株）社製）用インクタンクに充填し、実施例１で用いたバブルジェットプリンタのヘッドに装着した。また上記（１）で用意した基板もプリンタにセットし、ウェルに対してメトキシ基が加水分解されたシラン化合物を含むシランカップリング剤溶液を実施例８と同様にして供給した。この基板を２５℃、湿度１００％の恒温恒湿槽に３０分放置したのち、純水で洗浄、スピン乾燥し、その後１００℃で３０分間ベークして、各ウェルの底面にアミノ基を導入した。

【０１４０】（３）次にスクシイミジル－４－（マレイミドフェニル）ブチレート（アルドリッチ社製）を５ｗｔ％ＤＭＳＯ溶液に最終濃度が５ｗｔ％となるように溶解し、この溶液を上記（２）と同様にしてインクジェットプリンタで各ウェルに１００ｐｌづつ供給し、ついで３０℃、湿度１００％の恒温恒湿槽に基板を２時間放置した。次に基板を純粋で洗浄し、スピン乾燥させて各ウェルの底面にマレイミド基を導入した。

【０１４１】（４）ＤＮＡプローブとして５’末端の水酸基にリン酸基とヘキサメチレンを介してチオール基を結合した１８量体のオリゴマー（配列番号：１２）、配列番号１２のオリゴマーに対して１個のヌクレオチドがミスマッチのプローブ（配列番号：１３）、及び配列番号１２のオリゴマーに対して２個のヌクレオチドがミスマッチのプローブ（配列番号：１４）（全て日本製粉株式会社製、ＨＰＬＣグレード）を用意した。以下に配列番号：１２～１４の塩基配列とリンケージの構造を示す。

配列番号：１２

5'HS-(CH$_2$)$_6$-O-PO$_2$-O-TGTAAAACGACGGCCAGT3'

配列番号：１３

5'HS-(CH$_2$)$_6$-O-PO$_2$-O-TGTAAAACCACGGCCAGT3'

配列番号：１４

5'HS-(CH$_2$)$_6$-O-PO$_2$-O-TGTATAACCACGCCCAGT3'

（５）１０ｍＭのリン酸緩衝液に上記配列番号１２～１４の各々のＤＮＡプローブを最終濃度が１０μＭとなるように溶解させた。各々のＤＮＡプローブ溶液を上記実施例８と同様にして上記（３）で作成した基板のウェルに供給した。各ウェルを顕微鏡で観察したところ、供給されたＤＮＡプローブ溶液は、ウェルの開口部から盛り上がって存在しているが疎水性のマトリクスによってウェル内に止まっており、クロスコンタミネーションは観察されなかった。この基板を３０℃、湿度１００％の恒温恒湿槽に２時間放直し、その後純水で洗浄、スピン乾燥を行ない、各々のＤＮＡプローブのチオール基を各ウェルのマレイミド基と反応させ、ＤＮＡプローブを基板に結合させた。

【０１４２】（６）配列番号１２のＤＮＡプローブに対して完全相補的な一本鎖ＤＮＡを合成し、ＮａＣｌを５０ｍＭの濃度で含むＴＥ溶液に、この一本鎖ＤＮＡを最終浪度が１０μＭとなるように溶解した。この溶液に上記（５）で得たＤＮＡプローブ結合基板を浸漬し、８０℃～２５℃まで２時間かけて降温し、ハイブリダイゼーションを行なった。次にＮａＣｌを１０ｍＭの濃度で含むＴＥ溶液（ｐＨ８）を用いて２０℃で２０分間基板を洗浄したのち、スピン乾燥機で基板表面の洗浄液を除去した。

【０１４３】（７）ハイブリッドにインターカレートしてはじめて蛍光を発する試薬であるＹＯＹＯ－１をＮａＣｌを濃度５０ｍＭで含むＴＥ溶液に、最終濃度が１０

μMとなるように溶解した（ｐＨ８）。この溶液を上記（２）と同様にしてインクジェットプリンタを用いて上記（６）の処理を行なった基板の各ウェルに、１００ｐｌづつ供給し、実施例８と同様にして蛍光を観察定量した（B励起フィルターを使用）。なおＡｒｇｕｓ５０の信号増幅レベルは実施例８と同一である。

【０１４４】その結果、配列番号１４のＤＮＡプローブを結合させたウェルからは、バックグラウンドとほぼ同様の、１８００～２０００の蛍光強度が観察された。一方配列番号１２のＤＮＡプローブを結合させたウェルからは、７５００～８０００の蛍光強度が観察され、また配列番号１３のＤＮＡプローブを結合させたウェルからは、３１００～３３００の蛍光強度が観察された。更に固相をＴＥ緩衝液を用いて３５℃で１０分間洗浄して再度蛍光強度を測定したところ、配列番号１３のＤＮＡプローブを結合させたウェルからはバックグラウンドと同程度の蛍光強度しか観察されなくなった。

【０１４５】これらの結果から、本実施例に係るプローブアレイを用いることで、各ウェルにおいてハイブリダイゼーション反応を行なうことができ、更に配列番号９と完全相補的な標的核酸を特異的に検出できることが分かった。

【０１４６】実施例１２

（１）実施例１１と同様にして配列番号１２～１４のＤＮＡプローブを結合させた基板を用意した。

【０１４７】（２）配列番号１２～１４のＤＮＡプローブに対して完全相補的な３種類の一本鎖ＤＮＡを合成した。ＮａＣｌを５０mMの濃度で含むＴＥ溶液に上記３種類の一本鎖ＤＮＡを各々の濃度が１０μMとなる様に溶解した。なお各々の一本鎖ＤＮＡ溶液のｐＨは８である。バブルジェットプリンター（商品名：ＢＪＣ６２０；キヤノン（株）社製）用インクタンクを３個用意し、各々のインクタンクに上記の３種の一本鎖ＤＮＡ溶液を充填し、実施例１で用いたバブルジェットプリンタのヘッドに装着した。また上記（１）で用意した基板もプリンタにセットし、配列番号１２～１４のＤＮＡプローブが結合しているウェルに対して各々完全相補的な一本鎖ＤＮＡを含む溶液を１つのウェルにつき１００ｐｌづつ供給した。この時点で各ウェルの状態を顕微鏡で観察したところ、液のにじみ、クロスコンタミネーションは観察されず、またプローブアレイの各ウェルに個別に反応させるべき物質の溶液を供給できることが分かった。

【０１４８】（３）次に実施例１１と同様にして各ウェルにおいてハイブリダイゼーション反応を行なわせた後、実施例１１と同様にしてＹＯＹＯ－１溶液を各ウェルに供給し、蛍光を観察することでハイブリッドの検出を行なった。その結果、全てのウェルから７５００～８０００の強度の蛍光が観察された。このことから固相プローブアレイの各ウェルに個別に反応物質を供給し、各ウェルにおいてプローブと反応物質を反応させ、そして反応の結果物を検出できることが確認された。

【０１４９】実施例１３

（BM形成基板をエポキシ基導入用の溶液に浸漬してウェルにエポキシ基を導入した基板を用いたプローブアレイの製法）

（１）実施例７の（２）の記載に従ってブラックマトリックス付基板を作成した。

【０１５０】（２）実施例７の（１）の記載に従って、エポキシ基を結合したシラン化合物（γ－グリシドキシプロピルトリメトキシシラン）を含むシランカップリング剤（商品名：ＫＢＭ４０３；信越化学工業株式会社製）の１ｗｔ％水溶液を室温下で１時間攪拌して、該シラン化合物の分子内のメトキシ基を加水分解した。次いでこの溶液中に上記（１）で用意した固相を室温下で３０分間浸漬し、その後純水で該固相を洗浄し、窒素ガス流で水を除去し、１２０℃で５分間ベークし、ウェル底面にエポキシ基を導入した。この時点でBM表面の水に対する接触角は９５°と濡れにくい状態で有り、またウェル底部の水に対する接触角は３３°と濡れやすい状態であった。この様にBM形成後の固相をシランカップリング剤で処理することによってもウェル底面へのエポキシ基の導入は可能である。

【０１５１】（３）上記実施例８の（３）及び（４）に記載した方法に従って、配列番号：９～１１のＤＮＡプローブをウェルの底面に結合させた。

【０１５２】（４）配列番号９に対して相補的な塩基配列を有する一本鎖ＤＮＡをＤＮＡ自動合成橡で合成し、５’末端にヘキサノールアミンリンカーを介してテトラメチルローダミンを結合した標識化一本鎖ＤＮＡを得た。この標識化一本鎖ＤＮＡをＮａＣｌを５０mMの濃度で含むＴＥ溶液（ｐＨ８）に最終濃度が２μMとなるように溶解した。この溶液に上記（３）で得たＤＮＡプローブ結合基板を浸漬し、８０℃から２５℃まで２時間かけて降温してハイブリダイゼーション反応を行なった。その後プローブアレイを１０mM　ＮａＣｌ／ＴＥ緩衝液（ｐＨ８）を用いて２９℃で２０分間洗浄してプローブ核酸とハイブリダイズしなかった一本鎖ＤＮＡを洗い流した。次に実施例８と同様にして各ウェルからの蛍光量を定量した。

【０１５３】（５）結果

標識化一本鎖ＤＮＡと完全マッチである配列番号９のＤＮＡプローブを結合させたウェルからは８５００～９４００の蛍光量が確認された。また配列番号１０のＤＮＡプローブを結合させたウェルからは２８００～３４００の蛍光量が観察されまた配列番号１１のＤＮＡプローブを結合させたウェルからは１２００～１５００程度の蛍光量しか観察されなかった。また上記プローブアレイを１０mMＮａＣｌ／ＴＥ緩衝液（ｐＨ８）を用いて更に３５℃で１０分間洗浄したところ、配列番号１０のＤＮ

Ａプローブを結合させたウェルからの蛍光量は、バックグラウンドのレベルにまで低下した。よって本来施例にかかるプローブアレイを用いてもハイブリッドの標的物質の特異的な検出が可能であることが分かる。

【０１５４】

【発明の効果】以上説明した様に、本発明によればインクジェット技術を用いることによって固相上にプローブを含むスポットを、該プローブにダメージを与えることなしに、且つサテライトスポットを生じさせることなしにスポッティングすることができる。またこの方法を用いることによってプローブスポットを互いに独立に、且つ高密度に備えた高品質なプローブアレイを効率良く製造することができる。

【０１５５】更に本発明によれば少量の検体からでも標的物質に関するより多くの情報を、より正確に検査可能なプローブアレイを得ることができ、またそれを用いることでサンプル中に標的物質が存在するか否かをより正確、且つ迅速に判定できる。同様にこのプローブアレイを用いることでサンプル中の標的物質の構造をより正確に、且つ迅速に特定することができる。

【０１５６】また本発明によれば、プローブアレイの固相として表面にマトリクスパターンを形成し、ウェルを設けた固相を用いることで、固相へのプローブ溶液の供給、若しくは固相へのサンプルの供給の多少の位置ずれにも対処することができる。またマトリクスに種々の機能を担持させることで標的物質の検出、構造の特定等のより一層の高精度化が可能になった。

【０１５７】

【配列表】配列番号：１
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
他の情報：５’末端にチオール基が結合
配列
ACTGGCCGTCGTTTTACA
配列番号：２
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
他の情報：５’末端にチオール基が結合配列
ACTGGCCGTTGTTTTACA
配列番号：３
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
配列
ACTGGCCGCTTTTTTACA
配列番号：４
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
配列
ACTGGCATCTTGTTTACA
配列番号：５
配列の長さ：１２
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
配列
GCCTGATCAGGC
配列番号：６
配列の長さ：１０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
配列
AAAAAAAAAA
配列番号：７
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＰＮＡ
他の情報：Ｎ’末端にシステイン残基が結合
配列
ACTGGCCGTCGTTTTACA
配列番号：８
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＰＮＡ
他の情報：Ｎ’末端にシステイン残基が結合
配列
ACTGGCCGTTGTTTTACA
配列番号：９
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状

配列の種類：他の核酸　合成ＤＮＡ
他の情報：５’末端にアミノ基が結合
配列
TGTAAAACGACGGCCAGT
配列番号：１０
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
他の情報：５’末端にアミノ基が結合
配列
TGTAAAACCACGGCCAGT
配列番号：１１
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
他の情報：５’末端にアミノ基が結合
配列
TGTATAACCACGCCCAGT
配列番号：１２
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
他の情報：５’末端にチオール基が結合
配列
TGTAAAACGACGGCCAGT
配列番号：１３
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
他の情報：５’末端にチオール基が結合
配列
TGTAAAACCACGGCCAGT
配列番号：１４
配列の長さ：１８
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
他の情報：５‘末端にチオール基が結合
配列
TGTATAACCACGCCCAGT

【図面の簡単な説明】

【図１】バブルジェットヘッドを用いてプローブアレイを製造する方法の概略説明図である。

【図２】図１のバブルジェットヘッドのＡ－Ａ線断面図である。

【図３】実施例３においてバブルジェット法によってアルミ板上にスポッティングした核酸プローブの量の理論値と、実際の回収量とを対比するグラフである。

【図４】実施例４においてバブルジェット法によってアルミ板上にスポッティングした核酸プローブの量の理論値と、実際の回収量とを対比するグラフである。

【図５】（ａ）本発明にかかるプローブアレイの一実施態様の概略平面図である。

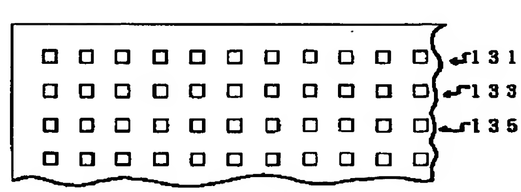
（ｂ）図５（ａ）のＢＢ線断面図である。

【図６】実施例８におけるスポッティング方法の説明図である。

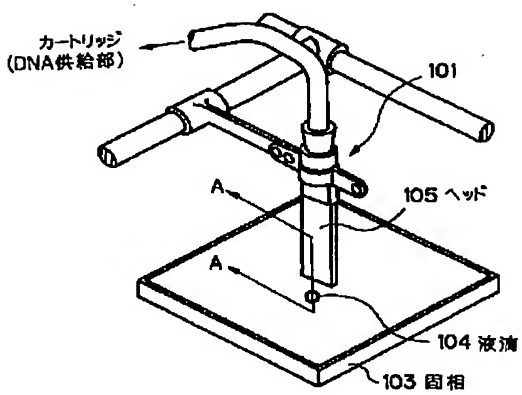
【符号の説明】

１０１　　ノズル
１０３　　固相
１０４　　液滴
１０５　　バブルジェットヘッド
１０７　　核酸プローブを含む吐出される液体
１０９　　保護膜
１１１－１、１１１－２　　電極
１１３　　発熱抵抗体層
１１５　　蓄熱層
１１６　　放熱性の良好なアルミナ等で形成されている基板
１１７　　発熱ヘッド
１１９　　吐出オリフィス
１２１　　メニスカス
１２３　　発泡領域

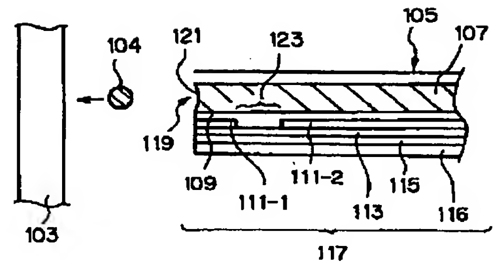
【図６】



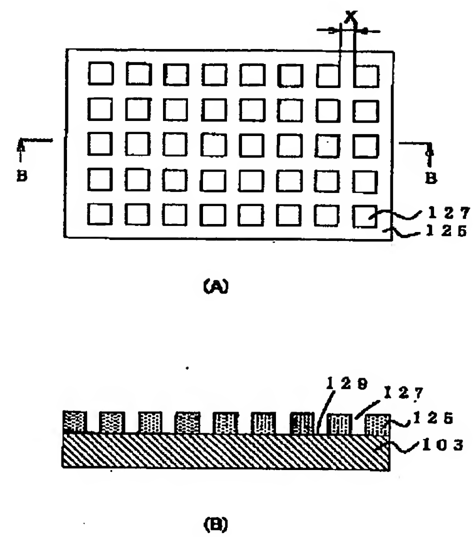
【図1】


カートリッジ
(DNA供給部)
101
A
105 ヘッド
A
104 液滴
103 固相

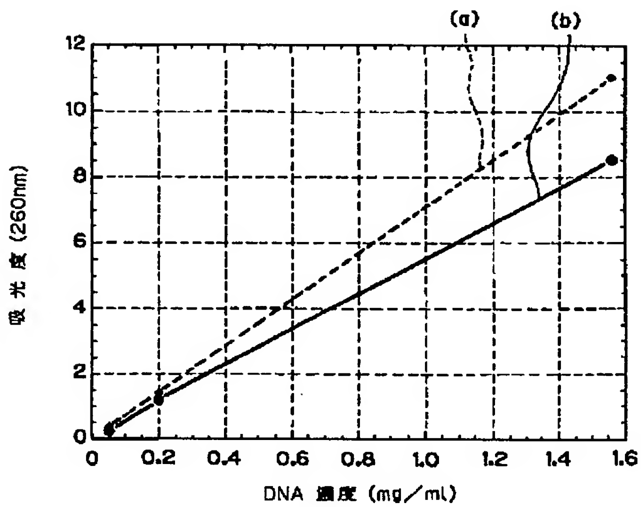
【図2】


104
103
121
123
105
107
119
109
111-1
111-2
113
115
116
117

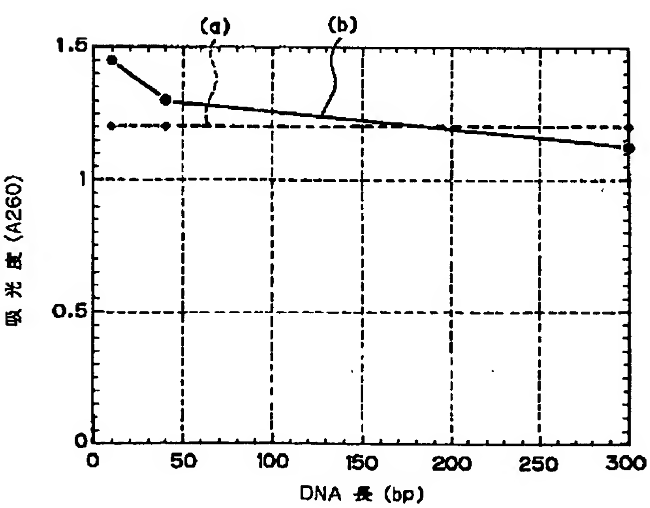
【図5】


X
B
B
127
125
(A)
129
127
125
103
(B)

【図3】


(a)
(b)
吸光度 (260nm)
DNA 濃度 (mg／ml)
(a)：理論値
(b)：測定値

【図4】


(a)
(b)
吸光度 (A260)
DNA 長 (bp)
(a)：理論値
(b)：測定値

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 11-187900
(43)Date of publication of application : 13.07.1999

(51)Int.Cl. C12Q 1/68
G01N 33/50
G01N 33/543
G01N 33/566
// C12N 15/09

(21)Application number : 10-209923
(22)Date of filing : 24.07.1998

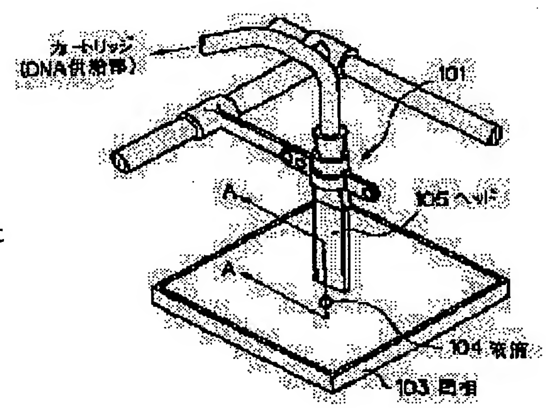
(71)Applicant : CANON INC
(72)Inventor : YAMAMOTO NOBUKO
OKAMOTO HISASHI
SUZUKI TOMOHIRO

(30)Priority

Priority number :	Priority date :	Priority country :
09207837	01.08.1997	JP
09287046	20.10.1997	JP

(54) METHOD FOR SPOTTING PROBE ONTO SOLID PHASE, PROBE ARRAY AND ITS PRODUCTION, AND DETECTION OF TARGET MATERIAL USING THE SAME, AND SPECIFICATION OF STRUCTURE OF TARGET MATERIAL

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method for spotting, in high density, a probe for detecting a target single-stranded nucleic acid, for determining base sequence, and so on, by supplying and attaching liquid which contains a probe which binds specifically to a target material onto solid phase surface by the ink jet method.
SOLUTION: This method comprises supplying liquid which contains a probe (e.g. singlestranded nucleic acid probe) which is capable of specifically binding to a target material to a bubble jet head 105 which is a kind of ink jet head and has a mechanism which gives thermal energy to the liquid and exhausts it, followed by exhausting and attaching the liquid onto the surface of a solid phase 103 such as a transparent glass plate as a droplet 104 by the ink jet method. This method allows spotting of a probe onto a solid phase to produce a probe array useful for detection of a target single-stranded nucleic acid and specification of base sequence of a target single-stranded nucleic acid.



LEGAL STATUS
[Date of request for xamination] 31.07.2000

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

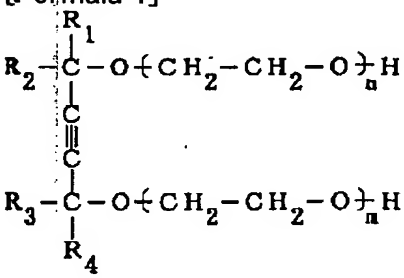
[Date of extinction of right]

* NOTICES *

CLAIMS

[Claim(s)]
[Claim 1] The spotting method to the solid phase of the probe characterized by having the process which the liquid which contains a combinable probe specifically to the target matter is supplied [process] to a solid phase front face by the ink-jet method, and makes it adhere to this solid phase front face.
[Claim 2] The spotting method according to claim 1 that this probe is a single strand nucleic-acid probe.
[Claim 3] The spotting method according to claim 2 that this single strand nucleic-acid probe contains a single stranded DNA probe.
[Claim 4] The spotting method according to claim 2 that this single strand nucleic-acid probe contains an RNA probe.
[Claim 5] The spotting method according to claim 2 that this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 6] It is the spotting method according to claim 2 which is that to which this solid phase front face and this single strand nucleic-acid probe have a functional group respectively, and these functional groups react by contact.
[Claim 7] The spotting method according to claim 6 that the functional group which this solid phase front face has is a maleimide machine, and the functional group which this single strand nucleic-acid probe has is a thiol (SH) machine.
[Claim 8] It is the spotting method according to claim 7 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 9] It is the spotting method according to claim 7 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 10] The spotting method according to claim 7 to which the maleimide machine on this glass substrate and the thiol group of this single strand nucleic acid are made to react at least for 30 minutes.
[Claim 11] The spotting method according to claim 10 that this single strand nucleic acid makes this maleimide machine and this thiol group react to an end for at least 2 hours or more including the single strand PNA probe which has a thiol group.
[Claim 12] The spotting method according to claim 11 that the thiol group of this single strand PNA probe end is what is introduced by combination of the cysteine by the side of the amino terminus of a single strand PNA probe.
[Claim 13] The spotting method according to claim 6 that the functional group which this solid phase front face has is an epoxy group, and the functional group which this single strand nucleic-acid probe has is an amino group.
[Claim 14] It is the spotting method according to claim 13 which this solid phase is a glass plate, and this epoxy group applies the silane compound which has an epoxy group in a molecule on the front face of this glass plate, and this compound and this glass plate are made to react, and is introduced.
[Claim 15] This epoxy group is the spotting method according to claim 13 introduced by the application of a up to [this solid phase of the polyglycidylmethacrylate which has an epoxy group].
[Claim 16] this liquid — this liquid — receiving — a urea — 5 - 10wt% and a glycerol — 5 - 10wt% and a thiodiglycol — 5 - 10wt% and acetylene alcohol — 1wt% — the included spotting method according to claim 1

[Claim 17] The spotting method according to claim 16 which is what has the structure by which this acetylene alcohol is shown by the following general formula (I).
[Formula 1]



(I)

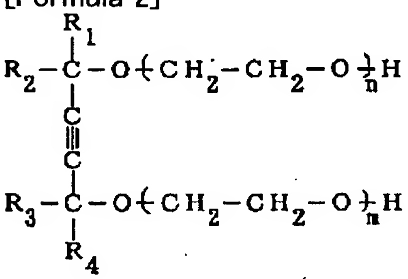
(R1, R2, R3, and R4 express an alkyl group among the above-mentioned formula, and m and n express an integer, respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)
[Claim 18] The spotting method according to claim 2 that the concentration of this single strand nucleic-acid probe in this liquid is 0.05-500microM.
[Claim 19] The spotting method according to claim 18 that the concentration of this single strand nucleic-acid probe in this liquid is 2-50microM.
[Claim 20] The spotting method according to claim 2 that the length of this single strand nucleic-acid probe is 2 - 5000 base length.
[Claim 21] The spotting method according to claim 20 that the length of this single strand nucleic-acid probe is 2 - 60 base length.
[Claim 22] The spotting method according to claim 1 that this ink-jet method is a bubble jet process.
[Claim 23] The spotting method according to claim 1 that this probe is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 24] The spotting method according to claim 1 that this probe is protein.
[Claim 25] The spotting method according to claim 24 that this protein is an antibody.
[Claim 26] The spotting method according to claim 24 that this protein is an enzyme.
[Claim 27] The spotting method according to claim 1 that this probe is an enzyme.
[Claim 28] The spotting method according to claim 1 of spotting this liquid by the density of 10000 or more per 1 square inch on this solid phase so that it may become the spot which became independent mutually.
[Claim 29] This solid phase is the spotting method according to claim 1 of having the surface characteristic with an evenly uniform front face.
[Claim 30] The spotting method according to claim 29 of spotting so that the interval of the adjoining spot may become more than the maximum width of this spot.
[Claim 31] The spotting method according to claim 30 blocked so that a nucleic acid may not adhere to parts other than a spot on this front face of solid phase.
[Claim 32] The spotting method according to claim 31 by which this blocking is attained by the cow serum albumin.
[Claim 33] The spotting method according to claim 1 that this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and supplies this liquid to each well.
[Claim 34] The spotting method according to claim 33 that this solid phase is optically transparent and this matrix is shading nature.
[Claim 35] The spotting method according to claim 33 that this matrix contains a resin.
[Claim 36] The spotting method according to claim 33 that the front face of this matrix is hydrophobic.
[Claim 37] The spotting method according to claim 33 that the base of this well is hydrophilic.
[Claim 38] The spotting method according to claim 33 that the thickness of this matrix is 1-20 micrometers.
[Claim 39] The spotting method according to claim 33 that the longest width of face of this well is 200 micrometers.
[Claim 40] The spotting method according to claim 33 that the width of face of this matrix is 1/2 of the longest width of face of this well - double precision.
[Claim 41] The probe array characterized by having the spot of the probe which has been independent mutually to two or more parts on the front face of solid phase by the density of 10000 or more pieces in a 1

square inch.
[Claim 42] This solid phase is a probe array according to claim 41 which has a flat front face and has the uniform surface characteristic.
[Claim 43] The probe array according to claim 42 this probe of whose is a single strand nucleic-acid probe.
[Claim 44] The probe array according to claim 43 in which this single strand nucleic-acid probe contains a single stranded DNA probe.
[Claim 45] The probe array according to claim 43 in which this single strand nucleic-acid probe contains a single-stranded-RNA probe.
[Claim 46] The probe array according to claim 43 in which this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 47] The probe array according to claim 43 which this single strand nucleic-acid probe has combined with this solid phase front face according to covalent bond by the reaction of the functional groups which this solid phase front face and a single strand nucleic-acid probe have respectively.
[Claim 48] The probe array according to claim 47 whose functional group which this single strand nucleic-acid probe has the functional group which this solid phase front face has is a maleimide machine, and is a thiol (SH) machine.
[Claim 49] The probe array according to claim 48 which this single strand nucleic-acid probe is a single strand PNA probe, and has a cysteine residue in an amino terminus side.
[Claim 50] The probe array according to claim 47 whose functional group which this single strand probe has the functional group which this solid phase front face has is an epoxy group, and is an amino group.
[Claim 51] The probe array according to claim 42 formed of grant of a up to [this solid phase of the liquid with which this spot contains a nucleic-acid probe].
[Claim 52] The probe array according to claim 42 this probe of whose is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 53] The probe array according to claim 42 this probe of whose is protein.
[Claim 54] The probe array according to claim 53 this protein of whose is an antibody.
[Claim 55] The probe array according to claim 53 this protein of whose is an enzyme.
[Claim 56] The probe array according to claim 42 this probe of whose is an antigen.
[Claim 57] The probe array according to claim 42 whose interval of this spot is more than the longest width of face of this spot.
[Claim 58] The probe array according to claim 41 each spot of whose this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and corresponds with the position of each well.
[Claim 59] The probe array according to claim 58 this probe of whose is a single strand nucleic-acid probe.
[Claim 60] The probe array according to claim 59 in which this single strand nucleic-acid probe contains a single stranded DNA probe.
[Claim 61] The probe array according to claim 59 in which this single strand nucleic-acid probe contains an RNA probe.
[Claim 62] The probe array according to claim 59 in which this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 63] The probe array according to claim 62 which this single strand nucleic-acid probe has combined with this solid phase front face according to covalent bond by the reaction of the functional groups which this solid phase front face and a single strand nucleic-acid probe have respectively.
[Claim 64] The probe array according to claim 63 whose functional group which this single strand nucleic-acid probe has the functional group which this solid phase front face has is a maleimide machine, and is a thiol (SH) machine.
[Claim 65] The probe array according to claim 64 which this single strand nucleic-acid probe is a single strand PNA probe, and has a cysteine residue in an amino terminus side.
[Claim 66] The probe array according to claim 63 whose functional group which this single strand probe has the functional group which this solid phase front face has is an epoxy group, and is an amino group.
[Claim 67] The probe array according to claim 58 formed of grant of a up to [this solid phase of the liquid with which this spot contains this nucleic-acid probe].
[Claim 68] The probe array according to claim 58 this probe of whose is the oligopeptide or polypeptide which

has a specific amino acid sequence.
[Claim 69] The probe array according to claim 58 this probe of whose is protein.
[Claim 70] The probe array according to claim 69 this protein of whose is an antibody.
[Claim 71] The probe array according to claim 69 this protein of whose is an enzyme.
[Claim 72] The probe array according to claim 58 this probe of whose is an antigen.
[Claim 73] The probe array according to claim 58 this matrix of whose is shading nature.
[Claim 74] This solid phase is a transparent probe array according to claim 73 optically.
[Claim 75] The probe array according to claim 58 in which this matrix contains a resin.
[Claim 76] The probe array according to claim 58 in which this probe has adhered only to this well.
[Claim 77] The probe array according to claim 58 whose thickness of this matrix is 1-20 micrometers.
[Claim 78] The probe array according to claim 58 whose longest width of face of this well is 200 micrometers.

[Claim 79] The probe array according to claim 58 whose intervals of this well are 1/2 of the longest width of face of this well - double precision.
[Claim 80] The probe array according to claim 41 which has at least two spots which consist of probes of a mutually different kind.
[Claim 81] The manufacture method of the probe array which is the manufacture method of the probe array which has the spot which contains a combinable probe independently specifically to the target matter in two or more parts on the front face of solid phase, and is characterized by having the process which makes the liquid containing this probe supply and adhere to the position on this front face of solid phase using the ink-jet method.
[Claim 82] The manufacture method according to claim 81 that this probe is a single strand nucleic-acid probe.
[Claim 83] The manufacture method according to claim 82 that this single strand nucleic-acid probe contains a single stranded DNA probe.
[Claim 84] The manufacture method according to claim 82 that this single strand nucleic-acid probe contains an RNA probe.
[Claim 85] The manufacture method according to claim 82 that this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 86] It is the manufacture method according to claim 82 which is that to which this solid phase front face and this single strand nucleic-acid probe have a functional group respectively, and these functional groups react by contact.
[Claim 87] The manufacture method according to claim 86 that the functional group which this solid phase front face has is a maleimide machine, and the functional group which this single strand nucleic-acid probe has is a thiol (SH) machine.
[Claim 88] It is the manufacture method according to claim 87 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 89] It is the manufacture method according to claim 87 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 90] The manufacture method according to claim 87 to which the maleimide machine on this glass substrate and the thiol group of this single strand nucleic acid are made to react at least for 30 minutes.
[Claim 91] The manufacture method according to claim 87 that this single strand nucleic acid makes this maleimide machine and this thiol group react to an end for at least 2 hours or more including the single strand PNA probe which has a thiol group.
[Claim 92] The manufacture method according to claim 91 that the thiol group of this single strand PNA probe end is what is introduced by combination of the cysteine by the side of the amino terminus of a single strand PNA probe.
[Claim 93] The manufacture method according to claim 86 that the functional group which this solid phase front face has is an epoxy group, and the functional group which this single strand nucleic-acid probe has is an amino group.
[Claim 94] It is the manufacture method according to claim 93 which this solid phase is a glass plate, and this epoxy group applies the silane compound which has an epoxy group in a molecule on the front face of this

glass plate, and this compound and this glass plate are made to react, and is introduced.
[Claim 95] This epoxy group is the manufacture method according to claim 93 introduced by the application of a up to [this solid phase of the polyglycidylmethacrylate resin which has an epoxy group].
[Claim 96] this liquid — this liquid — receiving — a urea — 5 - 10wt% and a glycerol — 5 - 10wt% and a thiodiglycol — 5 - 10wt% and acetylene alcohol — 1wt% — the included manufacture method according to claim 82
[Claim 97] The manufacture method according to claim 96 which is what has the structure by which this acetylene alcohol is shown by the following general formula (I).
[Formula 2]

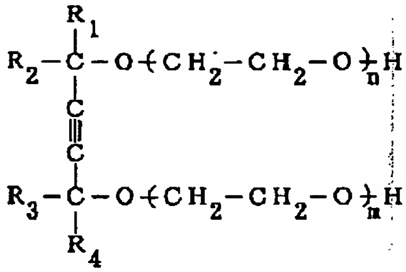


(I)

(R1, R2, R3, and R4 express an alkyl group among the above-mentioned formula, and m and n express an integer, respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)
[Claim 98] The manufacture method according to claim 82 that the concentration of this single strand nucleic-acid probe in this liquid is 0.05-500microM.
[Claim 99] The manufacture method according to claim 98 that the concentration of this single strand nucleic-acid probe in this liquid is 2-50microM.
[Claim 100] The manufacture method according to claim 82 that the length of this single strand nucleic-acid probe is 2 - 5000 base length.
[Claim 101] The manufacture method according to claim 100 that the length of this single strand nucleic-acid probe is 2 - 60 base length.
[Claim 102] The manufacture method according to claim 81 that this ink-jet method is a bubble jet process.
[Claim 103] The manufacture method according to claim 81 of spotting the liquid containing this single strand nucleic-acid probe by the density of 10000 or more per 1 square inch on this solid phase so that it may become the spot which became independent mutually.
[Claim 104] The manufacture method according to claim 81 that this probe is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 105] The manufacture method according to claim 81 that this probe is protein.
[Claim 106] The manufacture method according to claim 105 that this protein is an antibody.
[Claim 107] The manufacture method according to claim 105 that this protein is an enzyme.
[Claim 108] The manufacture method according to claim 81 that this probe is an antigen.
[Claim 109] This solid phase is the manufacture method according to claim 81 of having the surface characteristic with an evenly uniform front face.
[Claim 110] The manufacture method according to claim 109 which blocks so that a nucleic acid may not adhere to any parts other than the part by which this single strand nucleic acid is fixed, after making this single strand nucleic acid fix to solid phase.
[Claim 111] The manufacture method according to claim 110 of having the process to which this blocking dips the solid phase to which this single strand nucleic acid was fixed in bovine-serum-albumin solution.
[Claim 112] The manufacture method according to claim 111 that the concentration of this bovine serum albumin is 0.1 - 5%.
[Claim 113] The manufacture method according to claim 111 of performing being immersed [solution / bovine-serum-albumin / of this solid phase] for at least 2 hours.
[Claim 114] The manufacture method according to claim 109 of spotting so that the interval of the adjoining spot may become more than the maximum width of this spot.
[Claim 115] The manufacture method according to claim 81 that this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and supplies this liquid to each

well.
[Claim 116] The manufacture method according to claim 115 that this solid phase is optically transparent and this matrix is shading nature.
[Claim 117] The manufacture method according to claim 115 that this matrix contains a resin.
[Claim 118] The manufacture method according to claim 115 that the front face of this matrix is hydrophobic.
[Claim 119] The manufacture method according to claim 115 that the base of this well is hydrophilic.
[Claim 120] The manufacture method according to claim 115 that the longest width of face of this well is 200 micrometers.
[Claim 121] The manufacture method according to claim 115 that the width of face of this matrix is 1/2 of the longest width of face of this well – double precision.
[Claim 122] The manufacture method according to claim 115 that the thickness of this matrix is 1–20 micrometers.
[Claim 123] The manufacture method according to claim 115 which forms this matrix pattern by the photo lithography method.
[Claim 124] The manufacture method according to claim 123 of having the process which removes the pattern of this photoresist after this photo lithography method carries out patterning of this resin layer by using as a mask the pattern of process; which forms a resin layer in the 1st front face of this substrate, forms a photoresist layer on this resin layer, is exposed in the shape of a pattern, develops this photoresist layer so that it may correspond to this matrix pattern, and forms the pattern of a photoresist on this resin layer, and this photoresist.
[Claim 125] The manufacture method according to claim 123 of exposing in the shape of a pattern and having the process to develop so that this photo lithography method may form a photopolymer layer in the 1st front face of this substrate and may correspond this photopolymer layer to this matrix pattern.
[Claim 126] The manufacture method according to claim 125 that this photopolymer layer is a thing containing UV resist, a DEEP–UV resist, or ultraviolet–rays hardening resin.
[Claim 127] The manufacture method according to claim 126 that this UV resist is an cyclization polyisoprene–aromatic screw azide system resist, a phenol resin–aromatic azide compound system resist, or a novolak–resin–diazo naphthoquinone system resist.
[Claim 128] The manufacture method according to claim 126 that this DEEP–UV resist is a radiolysis type resist or a dissolution inhibitor system resist.
[Claim 129] this radiolysis type polymer resist — a polymethylmethacrylate, a polymethylene sulfone, poly hexafluoro butyl methacrylate, the poly methyl isopropenyl ketone, or bromination — the manufacture method according to claim 128 which is at least one chosen from a Polly 1–trimethylsilyl propyne
[Claim 130] The manufacture method according to claim 128 that this dissolution inhibitor system resist is cholic–acid o–nitrobenzyl ester.
[Claim 131] The manufacture method according to claim 126 that this DEEP–UV resist is the polyvinyl phenol –3, a 3'–diazide diphenyl sulfone, or a glycidyl methacrylate.
[Claim 132] The manufacture method according to claim 125 which carries out the postbake of the matrix pattern formed by patterning of this photopolymer layer further, and raises the water repellence of this matrix pattern.
[Claim 133] The manufacture method according to claim 115 which introduces into this solid phase front face the functional group which this probe has, and the functional group which can form covalent bond in advance of formation of this well.
[Claim 134] The manufacture method according to claim 115 which introduces into this solid phase front face the functional group which this probe has, and the functional group which can form covalent bond after formation of this well.
[Claim 135] The manufacture method according to claim 134 which gives the solution containing the compound for introducing this functional group into this solid phase front face to this well.
[Claim 136] The manufacture method according to claim 135 of performing grant to the well of this solution using the ink–jet method.
[Claim 137] The manufacture method according to claim 136 which is the silane coupling agent in which this solution contains the silane compound which has an epoxy group or an amino group in a molecule.
[Claim 138] The manufacture method containing the compound which this solution can react [compound] with the amino group on a glass substrate, and can make a maleimide machine introduce on a glass substrate

according to claim 136.
[Claim 139] The manufacture method according to claim 138 that this compound is N-maleimide KAPURO yloxy succinimide or SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate.
[Claim 140] Each spot and this sample of the probe array which has the probe specifically combined to the target matter which may be contained in the sample as two or more spots which became independent mutually on solid phase are contacted. In the method of detecting a reactant with this target matter and this probe, and detecting the existence of this target matter in this sample on this solid phase The method of detection of the target matter characterized by being formed when each of this spot spots the liquid containing this probe on solid phase by the ink-jet method.
[Claim 141] The method of detection according to claim 140 this probe of whose this target matter is the target single strand nucleic acid equipped with the predetermined base sequence, and is a target single strand nucleic-acid probe which has a complementary base sequence to this base sequence.
[Claim 142] The method of detection according to claim 141 with which this single strand nucleic-acid probe contains a single stranded DNA probe.
[Claim 143] The method of detection according to claim 141 with which this single strand nucleic-acid probe contains an RNA probe.
[Claim 144] The method of detection according to claim 141 with which this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 145] It is the method of detection according to claim 141 which is that to which this solid phase front face and this single strand nucleic-acid probe have a functional group respectively, and these functional groups react by contact.
[Claim 146] The method of detection according to claim 145 whose functional group which this single strand nucleic-acid probe has the functional group which this solid phase front face has is a maleimide machine, and is a thiol (SH) machine.
[Claim 147] It is the method of detection according to claim 146 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 148] It is the method of detection according to claim 146 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 149] The method of detection according to claim 146 to which the maleimide machine on this glass substrate and the thiol group of this single strand nucleic acid are made to react at least for 30 minutes.
[Claim 150] The method of detection according to claim 149 with which this single strand nucleic acid makes this maleimide machine and this thiol group react to an end for at least 2 hours or more including the single strand PNA probe which has a thiol group.
[Claim 151] The method of detection according to claim 146 whose thiol group of this single strand PNA probe end is what is introduced by combination of the cysteine by the side of the amino terminus of a single strand PNA probe.
[Claim 152] The method of detection according to claim 145 whose functional group which this single strand nucleic-acid probe has the functional group which this solid phase front face has is an epoxy group, and is an amino group.
[Claim 153] It is the method of detection according to claim 152 which this solid phase is a glass plate, and this epoxy group applies the silane compound which has an epoxy group in a molecule on the front face of this glass plate, and this compound and this glass plate are made to react, and is introduced.
[Claim 154] This epoxy group is the method of detection according to claim 152 introduced by the application of a up to [this solid phase of the polyglycidylmethacrylate resin which has an epoxy group].
[Claim 155] this liquid — this liquid — receiving — a urea — 5 - 10wt% and a glycerol — 5 - 10wt% and a thiodiglycol — 5 - 10wt% and acetylene alcohol — 1wt% — the included method of detection according to claim 141
[Claim 156] The method of detection according to claim 155 which is what has the structure by which this acetylene alcohol is shown by the following general formula (I).
[Formula 3]

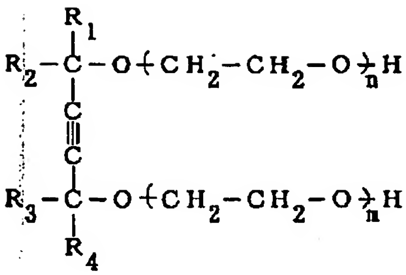


(I)

(R1, R2, R3, and R4 express an alkyl group among the above-mentioned formula, and m and n express an integer, respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)
[Claim 157] The method of detection according to claim 155 whose concentration of this single strand nucleic-acid probe in this liquid is 0.05-500microM.
[Claim 158] The method of detection according to claim 157 whose concentration of this single strand nucleic-acid probe in this liquid is 2-50microM.
[Claim 159] The method of detection according to claim 155 whose length of this single strand nucleic-acid probe is 2 - 5000 base length.
[Claim 160] The method of detection according to claim 159 whose length of this single strand nucleic-acid probe is 2 - 60 base length.
[Claim 161] The method of detection according to claim 141 this ink-jet method of whose is a bubble jet process.
[Claim 162] The method of detection according to claim 140 this probe of whose is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 163] The method of detection according to claim 140 this probe of whose is protein.
[Claim 164] The method of detection according to claim 163 this protein of whose is an antibody.
[Claim 165] The method of detection according to claim 163 this protein of whose is an enzyme.
[Claim 166] The method of detection according to claim 140 this probe of whose is an antigen.
[Claim 167] The method of detection according to claim 140 which spots this liquid by the density of 10000 or more per 1 square inch on this solid phase so that it may become the spot which became independent mutually.
[Claim 168] This solid phase is the method of detection according to claim 140 which has the surface characteristic with an evenly uniform front face.
[Claim 169] The method of detection according to claim 168 which spots so that the interval of the adjoining spot may become more than the maximum width of this spot.
[Claim 170] The method of detection according to claim 168 blocked so that a nucleic acid may not adhere to parts other than a spot on this front face of solid phase.
[Claim 171] The method of detection according to claim 170 with which this blocking is attained by the cow serum albumin.
[Claim 172] The method of detection according to claim 140 with which this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and supplies this liquid to each well.
[Claim 173] The method of detection according to claim 172 this matrix of whose this solid phase is optically transparent and is shading nature.
[Claim 174] The method of detection according to claim 172 with which this matrix contains a resin.
[Claim 175] The method of detection according to claim 172 with the hydrophobic front face of this matrix.
[Claim 176] The method of detection according to claim 172 with the hydrophilic base of this well.
[Claim 177] The method of detection according to claim 172 whose thickness of this matrix is 1-20 micrometers.
[Claim 178] The method of detection according to claim 172 whose longest width of face of this well is 200 micrometers.
[Claim 179] The method of detection according to claim 172 whose width of face of this matrix is 1/2 of the longest width of face of this well - double precision.
[Claim 180] The process which prepares the probe array which is the method of specifying the structure of

the target matter contained in a sample, and was equipped with the spot of the probe specifically combined with a solid phase front face to this specific matter; the specification-ized method of the structure of the target matter characterized by to have the process which detects combination with process; which contacts this sample at this spot and this target matter, and this probe.
[Claim 181] This specific matter is a target single strand nucleic acid, and the specification-ized structure is the base sequence of this target single strand nucleic acid. This probe array is equipped with two or more spots which contain respectively the single strand nucleic acid of a different base sequence on solid phase. at least one of the spots of these The spot of this plurality that the single strand nucleic acid of a complementary base sequence is included [and] is the specification-ized method according to claim 180 of making the liquid containing each single strand nucleic acid adhering on this solid phase using the ink-jet method, to the base sequence this target single strand nucleic acid is predicted to be.
[Claim 182] The specification-ized method according to claim 181 that this single strand nucleic-acid probe contains a single stranded DNA probe.
[Claim 183] The specification-ized method according to claim 181 that this single strand nucleic-acid probe contains an RNA probe.
[Claim 184] The specification-ized method according to claim 181 that this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 185] It is the specification-ized method according to claim 181 which is that to which this solid phase front face and this single strand nucleic-acid probe have a functional group respectively, and these functional groups react by contact.
[Claim 186] The specification-ized method according to claim 181 that the functional group which this solid phase front face has is a maleimide machine, and the functional group which this single strand nucleic-acid probe has is a thiol (SH) machine.
[Claim 187] It is the specification-ized method according to claim 186 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 188] It is the specification-ized method according to claim 186 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 189] The specification-ized method according to claim 186 to which the maleimide machine on this glass substrate and the thiol group of this single strand nucleic acid are made to react at least for 30 minutes.

[Claim 190] The specification-ized method according to claim 189 that this single strand nucleic acid makes this maleimide machine and this thiol group react to an end for at least 2 hours or more including the single strand PNA probe which has a thiol group.
[Claim 191] The specification-ized method according to claim 186 that the thiol group of this single strand PNA probe end is what is introduced by combination of the cysteine by the side of the amino terminus of a single strand PNA probe.
[Claim 192] The specification-ized method according to claim 185 that the functional group which this solid phase front face has is an epoxy group, and the functional group which this single strand nucleic-acid probe has is an amino group.
[Claim 193] It is the specification-ized method according to claim 192 which this solid phase is a glass plate, and this epoxy group applies the silane compound which has an epoxy group in a molecule on the front face of this glass plate, and this compound and this glass plate are made to react, and is introduced.
[Claim 194] This epoxy group is the specification-ized method according to claim 192 introduced by the application of a up to [this solid phase of the polyglycidylmethacrylate resin which has an epoxy group].
[Claim 195] this liquid — this liquid — receiving — a urea — 5 - 10wt% and a glycerol — 5 - 10wt% and a thiodiglycol — 5 - 10wt% and acetylene alcohol — 1wt% — the included specification-ized method according to claim 181
[Claim 196] The specification-ized method according to claim 195 which is what has the structure by which this acetylene alcohol is shown by the following general formula (I).
[Formula 4]


(I)

(R1, R2, R3, and R4 express an alkyl group among the above-mentioned formula, and m and n express an integer, respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)
[Claim 197] The specification-ized method according to claim 195 that the concentration of this single strand nucleic-acid probe in this liquid is 0.05-500microM.
[Claim 198] The specification-ized method according to claim 197 that the concentration of this single strand nucleic-acid probe in this liquid is 2-50microM.
[Claim 199] The specification-ized method according to claim 197 that the length of this single strand nucleic-acid probe is 2 - 5000 base length.
[Claim 200] The specification-ized method according to claim 199 that the length of this single strand nucleic-acid probe is 2 - 60 base length.
[Claim 201] The specification-ized method according to claim 181 that this ink-jet method is a bubble jet process.
[Claim 202] The specification-ized method according to claim 180 that this probe is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 203] The specification-ized method according to claim 180 that this probe is protein.
[Claim 204] The specification-ized method according to claim 203 that this protein is an antibody.
[Claim 205] The specification-ized method according to claim 203 that this protein is an enzyme.
[Claim 206] The specification-ized method according to claim 180 that this probe is an antigen.
[Claim 207] The specification-ized method according to claim 180 of spotting this liquid by the density of 10000 or more per 1 square inch on this solid phase so that it may become the spot which became independent mutually.
[Claim 208] This solid phase is the specification-ized method according to claim 180 of having the surface characteristic with an evenly uniform front face.
[Claim 209] The specification-ized method according to claim 208 of spotting so that the interval of the adjoining spot may become more than the maximum width of this spot.
[Claim 210] The specification-ized method according to claim 208 blocked so that a nucleic acid may not adhere to parts other than a spot on this front face of solid phase.
[Claim 211] The specification-ized method according to claim 210 that this blocking is attained by the cow serum albumin.
[Claim 212] The specification-ized method according to claim 180 that this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and supplies this liquid to each well.
[Claim 213] The specification-ized method according to claim 212 that this solid phase is optically transparent and this matrix is shading nature.
[Claim 214] The specification-ized method according to claim 212 that this matrix contains a resin.
[Claim 215] The specification-ized method according to claim 212 that the front face of this matrix is hydrophobic.
[Claim 216] The specification-ized method according to claim 212 that the base of this well is hydrophilic.
[Claim 217] The specification-ized method according to claim 212 that the thickness of this matrix is 1-20 micrometers.
[Claim 218] The specification-ized method according to claim 212 that the longest width of face of this well is 200 micrometers.
[Claim 219] The specification-ized method according to claim 212 that the width of face of this matrix is 1/2 of the longest width of face of this well - double precision.

[Translation done.]

* NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention relates to the method of spotting a probe to solid phase, a probe array, its manufacture method, the method of detection of the target single strand nucleic acid using the probe array, and the specification-ized method of the base sequence of a target single strand nucleic acid.
[0002]
[Description of the Prior Art] Use of the matter which can be specifically combined for example, with this target-nucleus acid as one of the technology which can perform quickly and correctly determination of the base sequence of a nucleic acid, detection of the target-nucleus acid in a sample, and identification of various bacteria, and the probe array which arranged many so-called probes in on solid phase is proposed.
[0003] the method of compounding the nucleic-acid probe on (1) solid phase as the general manufacture method of such a probe array, as indicated by the European Patent No. 373203 official report (EP 0373203B1), for example, and ** (2) — the method of supplying the nucleic-acid probe compounded beforehand on solid phase etc. is learned As advanced technology with which the detail of the above-mentioned method of (1) is indicated, for example, a U.S. Pat. No. 5405783 official report (USP5405783) is mentioned.
[0004] Moreover, the method of arranging cDNA in in the shape of an array, using micro pipetting as the method of the above (2) is indicated by a U.S. Pat. No. 5601980 official report (USP5601980), "a science (Science)", the 270th volume, 467 pages, and (1995), for example.
[0005] By the way, since the method of the above (1) is making the direct nucleic-acid probe compound on solid phase, it does not need to compound a nucleic-acid probe beforehand. However, it is difficult to refine the probe nucleic acid compounded on solid phase. It depends on the precision of the base sequence of a nucleic-acid probe for precision, such as sequencing of the nucleobase using the probe array, and detection of the target-nucleus acid in a sample, greatly. Therefore, improvement in the precision of a nucleic-acid probe is just going to ask for the further improvement as a process of a probe array with the more nearly quality method of the above (1).
[0006] On the other hand, the method of the above (2) can refine a nucleic-acid probe in advance of the combination to solid phase, while the synthetic step of a nucleic-acid probe is needed in advance of fixation in the solid phase of a nucleic-acid probe. In a present stage, it is considered by this reason as a process of a more nearly quality probe array for the method of the above (2) to be more desirable than the method of the above (1).
[0007] However, the technical problem of the method of the above (2) is in the method of spotting a nucleic-acid probe with high density to solid phase. For example, when performing base sequence determination of a nucleic acid using a probe array, it is desirable to arrange various nucleic-acid probes on solid phase as much as possible. Moreover, when detecting variation of a gene efficiently, it is desirable to arrange the nucleic-acid probe which has an array corresponding to each variation on solid phase. Furthermore, as for extraction of blood etc., in detection of the target-nucleus acid in a sample, and the variation of a gene and detection of a deficit, it is specifically desirable extraction of the sample from a subject and to stop to small quantity as much as possible, and it is desirable that the information on the base sequence of many in a small amount of sample as possible can therefore be acquired. When it thinks from these points, it is desirable that 10000 or more nucleic-acid probes are arranged at the probe array for example, at the 1 inch angle.

[0008]
[Problem(s) to be Solved by the Invention] As a result of examining many things under such a situation, this invention persons find out that a probe can be spotted very with high density using ink-jet regurgitation technology, and came to succeed in this invention.
[0009] And the purpose of this invention is efficiently extremely to offer the method of spotting correctly on solid phase, without doing an injury for the probe of a minute amount at this probe.
[0010] Moreover, other purposes of this invention are [rather than] about many information about a nucleic acid even from a small amount of sample to offer the probe array which can be inspected to accuracy.
[0011] Moreover, the purpose of further others of this invention is to offer the method of manufacturing efficiently, without damaging a probe for the probe array which the probe has combined on solid phase.
[0012] Furthermore, other purposes of this invention are to offer the method of detecting efficiently the target matter which may be contained in the sample.
[0013] Furthermore, other purposes of this invention are again to offer the specification-ized method of the structure of the target matter where the information about the structure of the target matter can be acquired even from a small amount of sample.
[0014]
[Means for Solving the Problem] The spotting method which takes like 1 operative condition as for this invention that the above-mentioned purpose can be attained is characterized by having the process which the liquid which contains a combinable probe specifically to the target matter is supplied [process] to a solid phase front face by the ink-jet method, and makes it adhere to this solid phase front face.
[0015] By using the spotting method concerning the above-mentioned mode, a probe can be given correctly and efficiently on solid phase, and a probe array can be manufactured efficiently.
[0016] Moreover, the probe array which takes like 1 operative condition as for this invention is characterized by having the spot of the probe which has been independent mutually to two or more parts on the front face of solid phase by the density of 10000 or more pieces in a 1 square inch. According to the probe array concerning the above-mentioned mode, many information can be acquired even from a small amount of sample from having the spot very with high density.
[0017] Moreover, the manufacture method of the probe array which takes like 1 operative condition as for this invention is the manufacture method of the probe array which has the spot which contains a combinable probe independently specifically to the target matter in two or more parts on the front face of solid phase, and is characterized by having the process which makes the liquid containing this probe supply and adhere to the position on this front face of solid phase using the ink-jet method. The probe array by which the spot has been arranged with high density can be manufactured efficiently, without harming a probe according to this mode.
[0018] Moreover, the method of detection of the target matter which takes like 1 operative condition as for this invention which can attain the above-mentioned purpose Each spot and this sample of the probe array which has the probe specifically combined to the target matter which may be contained in the sample as two or more spots which became independent mutually on solid phase are contacted. In the method of detecting a reactant with this target matter and a probe, and detecting the existence of this target matter in this sample on this solid phase Each of this spot is characterized by being formed by spotting the liquid containing this probe on solid phase by the ink-jet method. According to this mode, the target matter is efficiently detectable.
[0019] Furthermore, the specification-ized method of the structure of the target matter which takes like 1 operative condition as for this invention that the above-mentioned purpose can be attained The process which prepares the probe array which is the method of specifying the structure of the target matter contained in a sample, and was equipped with the spot of the probe specifically combined with a solid phase front face to this specific matter; The process which detects combination with process; which contacts this sample at this spot and this target matter, and this probe, It is characterized by ****(ing). When it is a single strand nucleic acid even from a small amount of sample by using this mode, the structure of the target matter, for example, the target matter, in this sample, the base sequence can be specified efficiently.
[0020] In addition, it is recognized if it is not appropriate for a USP No. 5601980 official report to use conventional ink-jet technology for spotting of a nucleic-acid probe. that is, the contamination of the spots of the nucleic-acid probe with which it is indicated that use of the ink jet printer technology of making little ink breathing out by the pressure wave (pressure wave) is not appropriate, the pressure wave for ink **** causes

a temperature rise with rapid ink temperature as the reason, and there is possibility do damage to a nucleic-acid probe, and spilling of the ink at the time of **** adjoins is caused in - of 31st line the 52nd line of the 2nd column of a USP No. 5601980 official report — danger is mentioned The drop of the liquid which contains a nucleic-acid probe at the nose of cam of a micropipette in a USP No. 5601980 official report using gas pressure on it is made to form, acting as the monitor of the size of this drop, when predetermined size is reached, pressure impression is stopped, and the method of supplying this drop on solid phase and manufacturing a probe array is indicated.

[0021] Moreover, the method of determining the base sequence of a target-nucleus acid as a USP No. 5474796 official report using forming the matrix of a hydrophobic property and a hydrophilic property in a solid phase front face, using piezo electric impulse jet pump equipment (Piezoelectric Impulse Jet Pump Apparatus) for the hydrophilic portion for four kinds of bases, being able to breathe out one by one, and manufacturing an oligonucleotide array in total and it is indicated.

[0022] However, these advanced technology is made to breathe out the nucleic-acid probe which has the base sequence of predetermined length beforehand using ink-jet technology, and it is not indicated at all about the technology of making a nucleic-acid probe arrange with high density and correctly.

[0023]

[Embodiments of the Invention] (Probe array process outline) Drawing 1 and drawing 2 are outline explanatory drawings of the manufacture method of of the probe array concerning this invention, for example, a nucleic-acid probe array. The liquid supply system (nozzle) which holds the liquid with which 101 contains in drawing 1 , the probe, for example, the nucleic-acid probe, as regurgitation liquid, possible [the regurgitation], the solid phase (for example, transparent glass plate etc.) with which, as for 103, this nucleic-acid probe should be combined, and 105 are bubble jet heads equipped with the mechanism in which this liquid is made to give and breathe out heat energy which are kinds of an ink-jet head. 104 is a liquid containing the nucleic-acid probe breathed out from the bubble jet head 105. Moreover, drawing 2 is the A-A line cross section of the bubble jet head 105 of drawing 1 , and the liquid with which 105 contains a bubble jet head and the nucleic-acid probe with which 107 should be breathed out in drawing 2 , and 117 are substrate portions which have the exoergic section which gives regurgitation energy to this liquid. The substrate portion 117 contains the base material 116 currently formed with the good alumina of the exoergic resistor layer 113 currently formed with the electrode 111-1,111-2 currently formed with the protective coat 109 currently formed by the silicon oxide etc., aluminum, etc., Nichrome, etc., the accumulation layer 115, and thermolysis nature etc.

[0024] The liquid 107 containing a nucleic-acid probe is coming to the regurgitation orifice (delivery) 119, and forms the meniscus 121 with the predetermined pressure. If an electrical signal joins an electrode 111-1,111-2 here, the field (foaming field) shown by 123 generates heat rapidly, a foam will be generated into the liquid 107 which has touched here, a meniscus will breathe out by the pressure, a liquid 107 will breathe out from an orifice 119, and it will fly toward the front face of solid phase 103. Although the amount of the liquid in which the regurgitation is possible changes with sizes of the nozzle etc. using a bubble jet head equipped with such structure, it is possible to control, for example to about 4-50 pico l., and it is very effective as a means to arrange a nucleic-acid probe with high density.

(Relation between regurgitation liquid and solid phase)

(Spot diameter on solid phase) In order to make it a value (it is a 1x106 individual grade as 10000 or more pieces and an upper limit for example, to 1 inch **) which described above the density on the solid phase of a nucleic-acid probe, as for the diameter of a spot of each nucleic-acid probe, it is desirable that the spot with it has been independent mutually. [that desirable and it is about 20-100 micrometers and] [mutual] And such a spot is determined by the property of the liquid breathed out from a bubble jet head, the surface characteristic of the solid phase to which this liquid adheres, etc.

[0025] (Property of regurgitation liquid) This liquid that a bubble jet head to the regurgitation was possible, and was breathed out from the head as a liquid for regurgitation reaches the position of the request on solid phase, and any liquids can be used if this nucleic-acid probe does not receive an injury further at the time of the mixed state with a nucleic-acid probe, and the regurgitation.

[0026] If it considers as the property of this liquid from a viewpoint of the regurgitation nature from a bubble jet head, 30 or more dyn/cm have [the viscosity] 1-15cps and surface tension desirable [and]. Moreover, when viscosity is made to 1-5cps and surface tension is made into 30 - 50 dyn/cm, the impact position on solid phase will become very exact, and it will be used especially suitably.

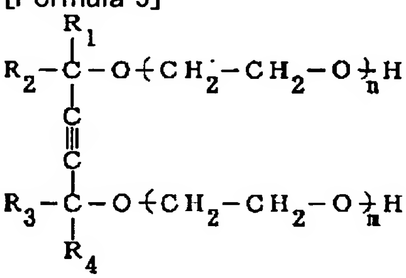
[0027] next — if the stability of the nucleic-acid probe at the time among the ink-jet regurgitation property

of this liquid and a liquid of the bubble jet regurgitation is taken into consideration — the inside of a liquid — for example, 2mer(s)- it is especially desirable 0.05-500microM and to make the nucleic-acid probe of 2mer-60mer contain by the concentration of 2-50microM especially 5000 mer

[0028] (Regurgitation liquid composition) As composition of the liquid breathed out from a bubble jet head It is what does not affect it substantially to a nucleic-acid probe as described above when it mixes with a nucleic-acid probe, and when it is made to breathe out from a bubble jet head. And if the conditions that the liquid composition in which the regurgitation is possible is desirable are normally fulfilled to solid phase using a bubble jet head Especially the liquid that contains the acetylene alcohol shown, for example by a glycerol, a urea, the thiodiglycol or ethylene glycol, isopropyl alcohol, and the following formula (I) although not limited is desirable.

[0029]

[Formula 5]



(I)

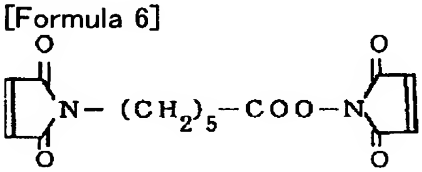
[0030] (R1, R2, R3, and R4 express the shape of a straight chain and the branched-chain alkyl group of carbon numbers 1-4 to an alkyl group and a concrete target among the above-mentioned formula (I), and m and n express an integer respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)

Furthermore, the liquid which contains 0.5 - 1wt% more preferably is suitably used 0.02 - 5wt% in the acetylene alcohol which a glycerol is shown by 5 - 10wt% 5 - 10wt%, and is specifically shown [urea] by the 5 - 10wt% and above-mentioned formula (I) in a thiodiglycol.

[0031] The configuration of the spot at the time of making the liquid which contains a nucleic-acid probe from a bubble jet head breathe out, and making it adhere on solid phase, when this liquid is used is circular, and when the breathed-out range does not spread and a nucleic-acid probe is spotted with high density, connection at the adjoining spot can be suppressed effectively. Furthermore, transformation of the nucleic-acid probe which it spotted on solid phase is not accepted, either. In addition, the property of the liquid used for manufacture of the nucleic-acid probe array of this invention is not limited to the above-mentioned thing. For example, when structure like a well which prevents mixing between the spots which the liquid given on solid phase with the bubble jet head spreads on this solid phase, and are adjoined is prepared in a solid phase front face, even if the viscosity and surface tension of a liquid, and also the base length and concentration of the above of a nucleic-acid probe are also out of range, it can use.

[0032] (Solid phase and kind of functional group of a nucleic acid) The position limited further is made to stop the spot of the nucleic-acid probe made to adhere on solid phase, and the method of making the both sides of a nucleic-acid probe and solid phase combine mutually the functional group in which a reaction is possible as a means by which it is effective in order to prevent contamination with the adjoining spot more certainly, and it is effective in combining a nucleic-acid probe firmly on solid phase is mentioned.

[0033] (A sulfhydryl group and maleimide machine) The example using the combination of for example, a maleimide machine and a thiol (-SH) machine as a desirable example is given. That is, a thiol (-SH) machine is combined with the end of a nucleic-acid probe, by processing so that a solid phase front face may have a maleimide machine, the thiol group of a nucleic-acid probe and the maleimide machine on the front face of solid phase which were supplied to the solid phase front face can react, a nucleic-acid probe can be fixed, and, as a result, the spot of a nucleic-acid probe can be formed in the position on solid phase. When the thing which the liquid of the composition which described above the nucleic-acid probe which has a thiol group was made to dissolve especially in an end is given to the solid phase front face which introduced the maleimide machine using the bubble jet head, a nucleic-acid probe solution forms a very small spot on solid phase. Consequently, a spot with a small nucleic-acid probe can be formed in the position on the front face of solid phase. In this case, the well which consists of a hydrophilic property and a hydrophobic matrix is formed in a

solid phase front face, and the need of preparing beforehand composition which prevents connection between spots is not accepted.
[0034] For example, the liquid adjusted so that it might become within the limits which the viscosity which contains the nucleic-acid probe of base length 18mer by the concentration M of 8micro, and surface tension described above a bubble-jet printer (tradename: -- BJC620; Canon [, Inc.], Inc. make --) However, the interval of the nozzle of solid phase and a bubble jet head is set as about 1.2-1.5mm using what was converted possible [printing] monotonously. When it is made to breathe out from this nozzle (discharge quantity is an about 24pico liter), on solid phase, a spot with a diameter of about about 70-100 micrometers can be formed. Moreover, visually, the spot (a "satellite spot" is called henceforth) originating in splash when a liquid reaches a solid phase front face was not accepted at all. Although the reaction of the maleimide machine on this solid phase and the sulfhydryl group of a nucleic-acid probe end is based also on the conditions of the liquid breathed out, it is completed in about 30 minutes under a room temperature (25 degrees C). In addition, this time is short as compared with the case where a piezo jet head is used for **** of a liquid. Although the reason is not clear, by the bubble jet process, it is thought that the temperature of the liquid which contains the nucleic-acid probe in a head by the principle rises, the reaction efficiency of a maleimide machine and a thiol group goes up as a result, and reaction time is shortened.
[0035] In addition, when using the combination of a maleimide machine and a thiol group, it is desirable to make the solution containing a nucleic-acid probe contain a thiodiglycol. Under neutrality and weak alkaline conditions, a thiol group forms a disulfide bond (-S-S-) and has a bird clapper in a dimer. However, a reactant fall with the thiol group and maleimide machine by dimer formation can be prevented by adding a thiodiglycol.
[0036] Although various methods can be used as the introductory method of the maleimide machine on the front face of solid phase, it is possible by, making an amino silane coupling agent react to a glass substrate for example, and making the reagent (EMCS reagent : product made from Dojin) containing N-(6-maleimide KAPURO yloxy) SUKUSHI imide (N-(6-Maleimidocaproyloxy) succinimide) shown with the amino group and following structure expression below react.
[0037]
[Formula 6]


[0038] Moreover, in case the nucleic-acid probe which the thiol group combined compounds DNA automatically for example, using a DNA automatic composition machine, it can be compounded by using 5'-Thiol-ModifierC6 (product made from Glen Research) as a reagent of a five prime end, and it is obtained by refining by the high performance chromatography after the usual deprotection reaction.
[0039] (The amino group and epoxy group) As combination of the functional group used for fixation, the combination of an epoxy group (on solid phase) and the amino group (nucleic-acid probe end) etc. is mentioned besides the combination of the above-mentioned thiol group and the above-mentioned maleimide machine. Introduction of the epoxy group on the front face of solid phase applies the polyglycidylmethacrylate which has an epoxy group to the solid phase front face which consists of a resin, or the silane coupling agent which has an epoxy group is applied to a glass solid phase front face, and glass, the method of making it react, etc. are mentioned.
[0040] As described above, when a functional group which reacts to a solid phase front face and the end of a single strand nucleic-acid probe mutually, and forms covalent bond is introduced, this nucleic-acid probe and solid phase are combined more firmly. Moreover, since a bonding site with the solid phase of this nucleic-acid probe can always be used as an end, the state of the nucleic-acid probe in each spot can be made uniform. As a result, the conditions of the hybridization of the nucleic-acid probe and target-nucleus acid in each spot will gather, and it is thought that the detection of a target-nucleus acid and the specification of a base sequence which improved further are attained. Furthermore, carrying out covalent bond of the nucleic-acid probe which the functional group attached to the end, and the solid phase can produce a probe array quantitatively compared with the nucleic-acid probe fixed on solid phase by noncovalent bonds (for example, electrostatic combination etc.), without producing the difference of the amount of combination of the probe DNA by the difference in an array or length. Furthermore, since all the base sequence portions of a nucleic

acid contribute to a hybridization reaction again, the efficiency of a hybridization reaction can be raised remarkably. Moreover, you may introduce an about one to seven-carbon number alkylene machine as a linker portion between the functional groups which participate in the reaction of the portion and solid phase which participate in hybridization with the target-nucleus acid of a single strand nucleic-acid probe. When combining a nucleic-acid probe with solid phase by this, a predetermined distance can be established between this solid phase front face and this nucleic-acid probe, and much more improvement in the reaction efficiency of a nucleic-acid probe and a target-nucleus acid can be expected.

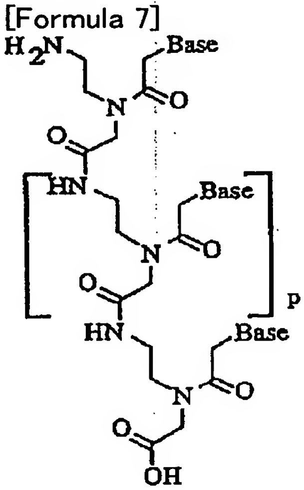
[0041] (Process of an array) One of the most desirable modes in the present condition of the manufacture method of the probe array which next starts this invention is explained. A liquid including acetylene alcoholic (for example, tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% of the structure shown by the above-mentioned general formula (I) is prepared thiodiglycol 7.5wt% urea 7.5wt% glycerol 7.5wt% as a liquid which distributes a nucleic-acid probe first. Next, the single strand nucleic-acid probe which the thiol group has combined with the end and about 2-5000 mers of whose length are about 2-60 mers especially, for example is compounded using a DNA automatic composition machine. subsequently, this nucleic-acid probe — in 0.05-500microM and the range which is 2-50microM especially, 1-15cps of concentration is mixed so that the viscosity of this liquid may be set to 1-5cps and 30 or more dyn/cm of surface tension may set it the above-mentioned liquid with 30 - 50 dyn/cm especially especially, and it considers as the liquid for regurgitation And it is filled up with this liquid for regurgitation in the nozzle of for example, a bubble jet head. Moreover, according to the above-mentioned method, the maleimide machine is introduced into solid phase on the front face. And the distance of the field and the nozzle side of a bubble jet head where the maleimide machine of this solid phase has combined this solid phase and this bubble jet head makes about 1.2-1.5mm approach, makes this bubble jet head drive, and makes this liquid breathe out. It is desirable to set it as a printing pattern which the spot on solid phase does not connect as regurgitation conditions here. For example, when it spots on the conditions of making the empty regurgitation carry out in the direction of 360dpi twice [after / the 1 time regurgitation], and making the empty regurgitation carry out in the direction of 720dpi after / the 1 time regurgitation / 5 times, when the resolution of the bubble jet head used for spotting is 360x720dpi, it is possible for the space between each spots to be set to about 100 micrometers, and to fully prevent contamination with the adjoining spot.

[0042] Subsequently, the reaction of the maleimide machine on solid phase and the thiol group of the nucleic-acid probe in a liquid progresses, and this solid phase is put for example, into a humidification chamber until this nucleic-acid probe is certainly fixed to solid phase. As described above, a room temperature (about 25 degrees C) is enough as this time at about 30 minutes. It is on solid phase after that, an unreacted nucleic-acid probe is flushed, and a nucleic-acid probe array is obtained.

[0043] After fixing this nucleic-acid probe to a solid phase front face here for the purpose of aiming at improvement in the detection precision in the case of performing detection of a target-nucleus acid etc. (S/N ratio), using this nucleic-acid probe array, it is desirable to block so that it may not combine with the target-nucleus acid with which the nucleic-acid probe uncombined portion of this solid phase is contained in a sample. Blocking is possible by dipping for example, this solid phase into 2% bovine-serum-albumin solution for about 2 hours, for example, or making the maleimide machine which has not been combined with the nucleic-acid probe on the front face of solid phase disassemble. For example, it is possible even if it uses DTT (dithiothreitol), beta-mercaptoethanol, etc. However, bovine-serum-albumin solution is most suitable, considering the effect which prevents adsorption of Indicator DNA. In addition, that what is necessary is just to carry out if needed, the process of this blocking performs supply to this probe array of a sample in limitation to each spot, and when there is no adhesion of the sample to parts other than a spot substantially, it does not need to perform it. Moreover, a well is beforehand formed in solid phase, and the process of blocking can be skipped when portions other than the well are processed so that a nucleic-acid probe cannot adhere easily.

[0044] Thus, you may constitute the probe array to produce so that it may have two or more spots which may constitute so that it may have two or more spots containing the same nucleic-acid probe, corresponding to the use, and contain respectively a nucleic-acid probe of a different kind. And the probe array by which the nucleic-acid probe has been arranged with high density by such method is used for detection of a target single strand nucleic acid, specification of a base sequence, etc. after that. for example, when the base sequence which may be contained in the sample uses for detection of a known target single strand nucleic acid The single strand nucleic acid which has a complementary base sequence to the base sequence of this

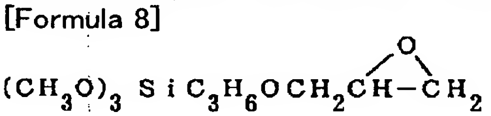
target single strand nucleic acid is used as a probe. The probe array by which two or more spots containing this probe are arranged on solid phase is prepared. After putting on the bottom of a condition which supplies a sample to each spot of this probe array, and this target single strand nucleic acid and a nucleic-acid probe hybridize at it, the existence of the hybrid in each spot is detected by known methods, such as fluorescence detection. The existence of the target matter in a sample is detectable with it. Moreover, in using for specification of the base sequence of the target single strand nucleic acid contained in the sample, two or more candidates of the base sequence of this target single strand nucleic acid are set up, and it spots to this solid phase by using as a probe the single strand nucleic acid which has a complementary base sequence respectively to this base sequence group. Subsequently, after putting on the bottom of a condition which supplies a sample to each spot and this target single strand nucleic acid and a nucleic-acid probe hybridize, the existence of the hybrid in each spot is detected by known methods, such as fluorescence detection. Thereby, the base sequence of a target single strand nucleic acid can be specified. Moreover, application to screening of the chemical which has the property combined with screening and DNA of the specific base sequence which DNA binding protein recognizes, for example as other uses of the probe array concerning this invention can be considered.
[0045] (Kind of ink-jet head) In the still more nearly above-mentioned explanation, although only the composition which performs grant to the solid phase of a nucleic-acid probe with a bubble jet head was explained, it is also possible to use the piezo jet head on which the liquid in a nozzle can be breathed out in this invention using ****** of a piezo-electric element, and it closes. However, since the ligation reaction to solid phase is completed for a short time and the secondary structure of DNA is also canceled by heat when a bubble jet head is used, as described above, a bubble jet head is an ink-jet head used more suitable for this invention at the point that the efficiency of the hybridization reaction following a degree can also be raised.
[0046] You may form two or more spots on solid phase simultaneously using the ink-jet head equipped with two or more heads so that the nucleic-acid probes contained between further two or more spots might differ.

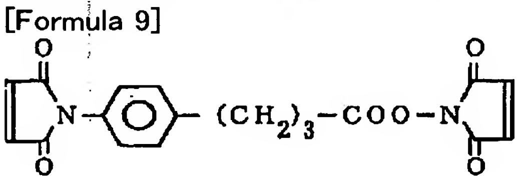
(PNA/DNA) this invention was explained so far, using a nucleic-acid probe as an example of a probe. As an example of a nucleic-acid probe, a deoxyribonucleic-acid (DNA) probe, a ribonucleic-acid (RNA) probe, and a peptide nucleic-acid (PNA) probe are included. PNA is an synthetic oligonucleotide which has the structure where four sorts of bases (an adenine, a guanine, a thymine, cytosine) contained in DNA combine with the peptide principal chain instead of a sugar-phosphate ester principal chain, and are shown in the following formula (II).
[0047]
[Formula 7]

(II)

[0048] ("Base" shows among a formula any of four kinds of bases (an adenine, a cytosine, a thymine, guanine) which constitute DNA they are.) Moreover, p expresses the base length of PNA.
PNA is compoundable by the method learned as for example, a tBOC type solid phase synthesis method or a Fmoc type solid phase synthesis method. and strong resistance [as opposed to enzymes, such as a nuclease and a protease, as compared with the oligonucleotide of nature / PNA /, such as DNA and RNA,] — having — the inside of a blood serum — enzyme-cleavage — almost — or it happens at all and is stable Moreover,

since it has neither the sugar part nor the phosphoric-acid machine, it is not necessary to adjust salt concentration at the time of hardly being influenced of the ionic strength of a solution, therefore making PNA and a target single strand nucleic acid react etc., and in order that there may be no still more nearly electrostatic rebounding, it is thought that the way of the hybrid of PNA and a target single strand nucleic acid is excellent in thermal stability as compared with the hybrid of a DNA probe and a target single strand nucleic acid or the hybrid of an RNA probe and a target single strand nucleic acid. And it is promising as a probe used for detection of a target-nucleus acid, or the determination of a base sequence from these properties. And the manufacture method of the nucleic-acid probe array concerning said this invention is effective when a PNA probe is applied as a nucleic-acid probe, and the PNA probe array by which the PNA probe has been arranged with high density and with high precision can be manufactured easily. Like a DNA probe or an RNA probe as a method of making the position where it was limited on solid phase specifically stopping a PNA probe, and attaining densification of a probe array Being able to use the method of introducing into each on the end of a PNA probe, and the front face of solid phase the functional group which has reactivity mutually, one of the desirable combination of a reactant basis is the combination of the thiol group (PNA end) same with having mentioned above, and a maleimide machine (solid phase front face). Introduction of the thiol group to a PNA end is attained by introducing the cysteine (CH(NH2) (COOH) CH2SH) machine which contains a thiol group in the amino terminus (equivalent to the five prime end of DNA) of for example, a PNA probe. Introduction of the cysteine to the amino terminus of a PNA probe can be performed by making the amino group of the amino terminus of for example, a PNA probe, and the carboxyl group of a cysteine react. Moreover, a cysteine can also be combined with the amino terminus of a PNA probe through a linker by making the amino group of the amino terminus of a PNA probe, and the carboxyl group of a suitable linker which has an amino group and the carboxyl group like for example, N2H(CH2)2O (CH2)2OCH2COOH react, and subsequently making the amino group of this linker, and the carboxyl group of a cysteine react. Thus, when a joint machine with solid phase is introduced through a linker, only a predetermined distance can make a reactive site with the target matter of a PNA probe estrange from solid phase, and much more improvement in hybridization efficiency is expected.
[0049] Moreover, in case PNA has a low case for some of the base length as compared with DNA of the base length with the same solubility over water and the liquid for ink-jet regurgitation is prepared, after dissolving PNA in trifluoroacetic acids (for example, 0.1wt% trifluoroacetic-acid solution etc.) etc. beforehand, it is desirable to prepare in the property which suits the ink-jet regurgitation using said various solvents. It is desirable to make it dissolve especially in a trifluoroacetic acid, when preventing the denaturation to the cystine by oxidization of the thiol group in the cysteine residue of a PNA end and aiming at much more improvement in the reaction efficiency of the thiol group of PNA, and the maleimide machine on the front face of solid phase. Moreover, although the reaction time of the thiol group introduced into the end of a DNA probe or an RNA probe and the maleimide machine on the front face of solid phase is enough in 30 minutes as described above (when a bubble jet head is used), even if it is the case where a bubble jet head is used in PNA, it is desirable to make it react for about 2 hours.
[0050] Furthermore, as a probe, it is not limited to a nucleic-acid probe, but the oligopeptide or polypeptide which has a combinable receptor and a specific amino acid sequence, a combinable oligopeptide and a combinable polypeptide, protein (for example, an antibody, an antigen, an enzyme, etc.), etc. can be used as a probe as specifically as a combinable ligand and a combinable ligand as specifically as the target matter in the sample used as the candidate for detection / analysis and the matter which can be combined specifically, for example, a receptor, In this case, the sulfhydryl group of the cysteine residue by which all are contained in protein can be used for a reaction.
[0051] According to the manufacture method of a probe array including the process which supplies a probe solution to solid phase using an ink-jet regurgitation process as explained above, a probe array can be formed very easily. When a functional group is introduced into each so that covalent bond may be especially formed between a nucleic-acid probe and a solid phase front face, the spots which adjoin even if it does not have a well etc. on a solid phase front face beforehand, namely, uses flat solid phase with a uniform surface characteristic (the ease of getting wet to water etc.) for it substantially do not connect. Moreover, the nucleic-acid probe array by which the nucleic-acid probe was arranged often [precision] and with high density as a result can be manufactured by the low cost very efficiently.
[0052] In addition, it does not eliminate at all that this uses the solid phase which equipped the front face with the well in this invention. For example, when the matrix pattern (a "black matrix" is called henceforth) of light

impermeability nature is beforehand formed between the wells to which a probe solution is supplied, the detection precision (SN ratio) of a case so that hybridization of the probe on solid phase and the target matter may be detected optically (for example, detection of fluorescence) can be raised further. Moreover, though a position gap of some arises in supply to the well of a probe solution when a front face establishes the low matrix of the compatibility over a probe solution between adjoining wells, a probe solution can be smoothly supplied to a desired well. You may use the solid phase which equipped the front face with the well for the purpose of using such an effect. The solid phase which has a matrix on a front face below, its manufacture method, and the operation in this embodiment of this solid phase are explained.
[0053] An example of the probe array in this mode is shown in drawing 5 . Drawing 5 (A) is a plan and drawing 5 (B) is the BB cross section. This probe array has the structure which formed the matrix pattern 125 which has the frame structure in which the crevice (well) 127 arranged in the shape of a matrix was formed on solid phase 103. The well 127 mutually isolated by the matrix 125 (heights) was formed as a breakthrough in a matrix pattern (Japanese common chestnut omission section), and the side attachment wall consists of heights, and is in the state where the front face of solid phase 103 was exposed to the base 129. The amount of [of solid phase 103] surface outcrop forms the front face in which a probe and combination are possible, and the probe (un-illustrating) is being fixed to the predetermined crevice.
[0054] When the method of measuring and detecting the fluorescence to which detection, for example, a reactant, emits the reactant of a probe and the target matter optically is used for it, and improvement in detection sensitivity, a S/N ratio, and reliability is taken into consideration, as for the material which forms a matrix pattern, what has shading nature is desirable. As such a material, metals (chromium, aluminum, gold, etc.), a black resin, etc. are mentioned, for example. As a resin of this black, the thing which made the black color and the black pigment contain is mentioned to resins, such as an acrylic, a polycarbonate, polystyrene, polyethylene, a polyimide, an acrylic-acid monomer, and urethane acrylate, and photosensitive resins, such as a photoresist. As an example of a photopolymer, UV resist, a DEEP-UV resist, ultraviolet-rays hardening resin, etc. can be used, for example. As a UV resist, positive resists, such as negative resists, such as an cyclization polyisoprene-aromatic screw azide system resist and a phenol resin-aromatic azide compound system resist, and a novolak-resin-diazo naphthoquinone system resist, can be mentioned.
[0055] as a DEEP-UV resist — as a positive resist — for example, a polymethylmethacrylate, a polymethylene sulfone, poly hexafluoro butyl methacrylate, the poly methyl isopropenyl ketone, and bromination — dissolution inhibitor system resists, such as radiolysis type polymer resists, such as a poly 1-trimethylsilyl propyne, and cholic-acid o-nitrobenzyl ester, etc. can be mentioned, and the polyvinyl phenol -3, a 3'-diazide diphenyl sulfone, polymethacrylic-acid glycidyl, etc. can be mentioned as a negative resist
[0056] As ultraviolet-rays hardening resin, the polyester acrylate which is chosen from oxime system compounds, such as a benzophenone and its substitution derivative, a benzoin and its substitution derivative, an acetophenone and its substitution derivative, and a benzyl, etc. and which contained one sort or two sorts or more of photopolymerization initiators about 2 to 10% of the weight, epoxy acrylate, urethane diacrylate, etc. can be mentioned. Carbon black and a black organic pigment can be used as a black pigment.
[0057] In addition, when not detecting the reactant of a probe and the target matter optically, or when the light from a matrix does not affect optical detection of a reactant, using the object of un-shading nature as a matrix pattern formation material is not barred at all.
[0058] Next, the method of carrying out the coat of the photoresist on the resin metallurgy group which carried out the coat to the substrate front face as one method of forming a matrix pattern using material which was described above, and carrying out patterning of the resin according to processes, such as etching, after patterning is mentioned. Moreover, if it is a photosensitive resin, it is also possible by hardening the resin itself exposure, development, and if needed according to the process of the photo lithography using the photo mask to carry out patterning.
[0059] When a matrix 125 is made into the product made of a resin here, the front face of a matrix 125 becomes hydrophobic. This composition is desirable when using the solution of a drainage system as a solution containing the probe supplied to a well. That is, though a probe solution is supplied with a position gap of some in case a probe solution is used for a well and the ink-jet method is supplied to it, a probe solution will be supplied to a desired well very smoothly. moreover — the case where the probe of a different kind is supplied between the wells which adjoin simultaneously — these wells — between different probe solutions supplied in between — being mixed (cross contamination) — protecting also becomes possible
[0060] Usually, since the probe solution of living body related substances, such as a peptide and a nucleic

acid, is a solution of a drainage system in many cases, in such a case, a matrix pattern can use the composition of water repellence and a bird clapper suitably.
[0061] Next, how to consider the base (outcrop on the front face of solid phase) of a well as the composition in which a probe and combination are possible is explained. The functional group made to hold on the base of a well changes with combination with the functional group which a probe is made to support. For example, when using the nucleic-acid probe which introduced the thiol group into the end as a probe, the thiol group of the nucleic-acid probe supplied to the well by introducing a maleimide machine into a solid phase front face as mentioned above forms the maleimide machine and covalent bond on the front face of solid phase, and a nucleic-acid probe is fixed to a solid phase front face. To the nucleic-acid probe which has an amino group at the nucleic-acid probe end similarly, introduction of the epoxy group on the front face of solid phase is desirable. As other combination of such a functional group, introduction of the amino group on the front face of solid phase is desirable to the nucleic-acid probe which has a carboxyl group (based on the introduction to the nucleic-acid probe end of a succinimide derivative) at the end, for example. Although the fixing nature to the solid phase top at the time of the combination of this amino group and an epoxy group breathing out a nucleic-acid probe solution by the ink-jet regurgitation method as compared with the combination of a thiol group and a maleimide machine is not good, when the well is prepared in solid phase, it is the thing of the grade which can be disregarded.
[0062] The introduction to the solid phase front face of the amino group or an epoxy group As mentioned above, in using a glass plate as solid phase After processing this glass-plate front face with alkali, such as a potassium hydroxide and a sodium hydroxide, first and exposing a hydroxyl group (silanol group) on a front face, The amino group The silane coupling agent containing the silane compounds (for example, gamma-glycidoxypropyltrimetoxysilane etc.) which introduced the introduced silane compounds (for example, N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane etc.) and the epoxy group It can carry out by making it react with the hydroxyl group on this front face of a glass plate. Moreover, a maleimide machine can introduce N-maleimide KAPURO yloxy succinimide, SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate, etc. into a glass-plate front face by making it react with this amino group, after introducing the amino group into a glass-plate front face by the above-mentioned method.
[0063] In addition, the structure of N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane, gamma-glycidoxypropyltrimetoxysilane, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate is as follows.
** N-beta (aminoethyl)-gamma-aminopropyl trimethoxysilane : (CH3O) it is 3H6NHC2H4NH2** gamma-glycidoxypropyltrimetoxysilane: [0064] 2 SiC.
[Formula 8]


[0065] ** SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate : [0066]
[Formula 9]


[0067] When an epoxy group is introduced into a solid phase front face in the surface treatment of the above-mentioned solid phase, after combining this epoxy group and a probe, the base of a well can be made into a hydrophilic property by carrying out ring breakage of the unreacted epoxy group using ethanolamine solution etc., and changing into a hydroxyl group. This operation is desirable when supplying this probe and the drainage system solvent containing the target matter which reacts specifically to the well which combined the probe.
[0068] Moreover, when using a resin substrate as solid phase, a hydroxyl group, a carboxyl group, or the amino group can be introduced into a resin substrate front face by the method of the publication of Chapter 5 of Organic Thin Films and Surface, Vol.20, and Academic Press. Or it is also possible to introduce the amino group and an epoxy group or to introduce a maleimide machine further using the silane compound which has an amino group and an epoxy group like the case of the glass plate described above after introducing a

hydroxyl group by this method. By the way, introduction of the functional group to the above-mentioned solid phase may be performed before formation of a matrix on a solid phase front face, or you may perform it after matrix formation. What is necessary is just to supply a reaction solution to each well by the ink-jet method etc. that what is necessary is just to supply a reaction solution required for introduction of a functional group to a solid phase front face on a solid phase front face by the method of a spin coat, a DIP coat, etc. if it is before matrix formation, if it is after matrix formation.
[0069] Moreover, oxidize a resin substrate front face, introduce a hydroxyl group, the silane coupling agent and this hydroxyl group containing the silane compound which subsequently has an amino group are made to react, the amino group is introduced, and the method to which this amino group and the functional group of a probe are made to react is mentioned as indicated by JP,60-015560,A as a method of combining a probe with a resin substrate.
[0070] Moreover, when the substrate after pretreatment is a hydrophilic property, the resin for the matrix formation made of a resin in which the matrix pattern of another side is formed and which spread a water-repellent material previously relatively can be used as it is. Moreover, when the further water repellence is needed, it can also add a water repellent in matrix material. Moreover, when a matrix pattern is formed by photopolymers, such as a photoresist, it is also possible by performing a postbake on suitable conditions after exposure and development to give strong water repellence with a matrix pattern.
[0071] Although the case where a probe solution was a hydrophilic property was described when saying so far which it was, what is necessary will be just to carry out reverse processing in a lipophilic case [a probe solution].
[0072] The size and the configuration of a well of a matrix pattern can be suitably chosen with the supply methods, such as size of a substrate, size of the whole array finally produced, the number of probe kinds that constitutes an array or the formation method of a matrix pattern, and a probe solution to a matrix pattern gap, the method of detection, etc.
[0073] A square configuration's can be alike, in addition the cross section of a field parallel to the substrate shown in drawing 5 as a configuration can consider as various configurations, such as a rectangle, various polygons, circular, and an ellipse form.
[0074] As size of a well, when the size of the number of reaction kinds and the whole array is taken into consideration, the longest width of face of 300 micrometers or less is desirable. For example, as shown in drawing 5 , when making the cross section in a direction parallel to the substrate of a well into a square, the length of one side can be set to 200 micrometers or less. Furthermore, in making a well into a rectangle, when making circular 200 micrometers or less of the long side, it is more desirable to set the diameter to 200 micrometers or less. The minimum of the size can be set to about 1 micrometer.
[0075] The array gestalt of each well can be suitably changed according to requests, such as a mode which is the vertical direction in a plan like drawing 5 , and is arranged at equal intervals, and a mode which shifts and arranges the position of a well in an adjacent train.
[0076] As for the distance between adjoining wells, it is desirable to set it as an interval which it does not make produce cross contamination even if a position gap of some arises between a regurgitation position and the well which should be supplied, in case a probe solution is supplied to a well for example, by the ink-jet method. Moreover, when the size etc. and cross contamination of the whole array, and the operability in the case of supply of various solutions are taken into consideration, it is desirable that the distance between adjoining wells is in the range of 1/2 of the longest width of face of a well - double precision.
[0077] For example, if the size of a substrate is made into a suitable size (1 inch x1 inch or 1cmx1cm) by the case where a well is made into a square configuration when automating operation of probe fixation, sample supply, detection, etc. From the need of fully achieving the function as combinatorial chemistry to 100 pieces x 100 pieces Or since it is desirable for the probe kind beyond 1000 piece x1000 piece to exist, it is desirable to set to 200 micrometers or less distance between the wells which adjoin one side of the square configuration of a well 1-200 micrometers also in consideration of the size of the matrix itself.
[0078] Moreover, although the thickness (height from a solid phase front face) of a matrix is determined in consideration of the capacity of the formation method of a matrix pattern, or a well, the amount of the probe solution to supply, etc., it is desirable to be preferably referred to as 1-20 micrometers. By considering as such thickness, namely, when [for example,] a probe solution is supplied to each well using an ink-jet regurgitation method, Even if it is the case where it can adjust only to a difficult property that this probe solution forms a small spot for the property of a probe solution on this solid phase front face, in relation with

ink-jet regurgitation conditions The position on solid phase can be made to be able to stop this probe solution, and cross contamination can be prevented very effectively.

[0079] Temporarily, the capacity of the well in the upper limit of the above-mentioned desirable range becomes 200micrometerx200micrometerx20micrometer, i.e., 800pl. Moreover, if distance between adjoining wells (x of drawing 1) is similarly set to 200 micrometers in this size, 625 well densities /of 2 will be obtained cm. That is, the array which has the well density of two or more [102 //cm] as order is obtained. Moreover, if a well is made into the square configuration whose one side is 5 micrometers, and distance between adjoining wells is also set to 5 micrometers and sets thickness of a matrix pattern to 4 micrometers, the capacity of a well will serve as 0.1pl(s) and 1 million [/] of the number will be set to 2 cm. since 5micrometerx5micrometerx4micrometer patterning is realistic size in the present ultra-fine processing technology — as order — the well of two or more [106 //cm] — the array of density may also serve as the range of invention of this invention

[0080] When a probe solution or a probe, and the supply volume to the well of the matter which should react consider as the amount of said mostly with the capacity of a well in this mode, it becomes a 0.1pico liter (pl) to a 1nano liter (nl) from the above-mentioned calculation in general. Moreover, when it considers as non-compatibility to the solution to which a matrix is supplied, depending on ****, it becomes possible to stop the liquid of the amount which exceeds the capacity of a well with the surface tension in the opening upper part of a well. In such a case, for example, 10 times of a well, volume 10 times the number of this can be supplied, and it can be made to hold. That is, the liquid of number 10nl will be supplied from Number pl. It is desirable in any case, to supply a probe solution at a well using the ink-jet method which can supply position precision and amount-of-supply precision good, although a general micro dispenser and a general micropipette are also possible for the supply to such a little well of liquid. On an ink-jet print, since it positions with high precision to mum order and the regurgitation of the ink is carried out, it can be told to supply of the solution to a well that it is extremely suitable. moreover, the amount of the ink breathed out — general — several — from pl, since it is number 10nl, it can be said that it is suitable for supply of the solution to a well also at this point

[0081] If a drop adheres to the field containing a well even if according to this mode the breadth of a drop is quantitatively controlled by the reaction and well on a nucleic-acid probe and the front face of solid phase and disorder of some is in a discharge direction, the portion will be crawled and the portion concerning the matrix of a drop will be smoothly contained in a well, when the matrix serves as non-compatibility to regurgitation liquid.

[0082] Although especially the ink-jet method used for this invention is not restricted, a piezo jet process, the bubble jet process using thermal foaming, etc. can be used, for example.

[0083] By the way, what can form a matrix in a front face in the 2nd mode further is [that what is necessary is just what can introduce various functional groups which were described above on the solid phase front face as a material which can be used as solid phase 103 in this invention] desirable. And when constructing the detection system which minded solid phase when the reactant of a probe and the target matter was detected optically, it is desirable to make solid phase into transparent solid phase optically. The glass which contains synthetic quartz, a fused quartz, etc. as such a material, silicon, acrylic resin, polycarbonate resin, polystyrene resin, vinyl chloride resin, etc. are mentioned. Moreover, when detecting optical detection of this reactant without minding solid phase, it is desirable to use black solid phase optically, and the resin substrate containing black stain pigments, such as carbon black, etc. is used.

[0084] In this invention, the solution of the matter which should react to these probes array is supplied, and it reacts by putting on a suitable reaction condition. When the solution of matter which is different in an individual well and which should react needs to be supplied, at least one sort of solutions which at least one sort of matter which should be made to react to a probe dissolved in each of two or more wells of a probe array are supplied. In this case, supply of the quantitive liquid without cross contamination which limited the supply field when it was affinity-like to the well with which the probe of a probe array with which the solution supplied is already formed is combined and was un-keeping-good relations-like [a matrix pattern] is attained as mentioned above. Since many of living body-related matter is water-soluble like the matter shown in Table 1, in a well, in this case, a hydrophilic property and a matrix pattern serve as water repellence. Moreover, as mentioned above, if the ink-jet method is used also for supply of these matter that should react, supply will become possible quantitatively about minute amount volume at it.

[0085] Since the volume of the probe supplied in this invention in order to combine with a substrate, or the volume of the matter which should react is a minute amount, it is desirable to include the conditions from

which both reaction conditions protect evaporation of the supplied solution and ****.
[0086] this invention is explained still in detail with an example below.
[0087] Example 1 (the process of the nucleic-acid probe array using the bubble-jet printer, and evaluation of the probe array)
(1) The glass plate of the 1 inch angle of substrate washing was put into the rack, and it dipped in the detergent for ultrasonic cleaning overnight. Then, ultrasonic cleaning was performed for 20 minutes in the detergent, and rinsing removed the detergent after that. It was distilled water, and after rinsing, it ultrasonicated for 20 minutes further in the container containing distilled water. Next, the glass plate was dipped in 1-N sodium-hydroxide solution beforehand warmed at 80 degrees C for 10 minutes. Rinsing and distilled water washing were performed succeedingly and the glass plate for probe arrays was prepared.
[0088] (2) The 1wt% solution of the silane coupling agent (tradename : KBM603; Shin-Etsu Chemical [Co., Ltd.] Co., Ltd. make) containing the silane compound (N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane) which combined the surface treatment amino group was stirred under the room temperature for 2 hours, and the methoxy machine in the molecule of the above-mentioned silane compound was hydrolyzed. Subsequently, after dipping the substrate obtained above (1) in this solution for 20 minutes at a room temperature (25 degrees C), it pulled up and both sides of a glass plate were made to spray and dry nitrogen gas. Next, for 1 hour, silane coupling processing was completed, and the amino group was introduced into the substrate front face. [in the oven which heated the glass plate at 120 degrees C] Subsequently, 2.7mg weighing capacity of the N-maleimide KAPURO yloxy succinimide (product made from N-(6-Maleimidocaproyloxy) succinimide;Dojin) (it abbreviates to EMCS henceforth) was carried out, and the EMCS solution which dissolved so that the last concentration might become 1:1 solutions of dimethyl sulfoxide (DMSO)/ethanol in ml and 0.3mg /was prepared. The glass plate which performed silane coupling processing was dipped in this EMCS solution at the room temperature for 2 hours, and the amino group currently supported by silane coupling processing on the glass-plate front face and the carboxyl group of an EMCS solution were made to react. The maleimide machine of the EMCS origin will exist in a glass-plate front face on a front face in this state. After washing the glass plate pulled up from the EMCS solution one by one by the mixed solvent of DMSO and ethanol, and ethanol, it was dried under nitrogen-gas-atmosphere mind.
[0089] (3) The single strand nucleic acid of the array number 1 was compounded using the synthetic DNA automatic composition machine of probe DNA. In addition, the thiol (SH) machine was introduced by using a thiol modifier (Thiol-Modifier) (grain research (GlenResearch) company make) for the single stranded DNA end of the array number 1 at the time of composition with a DNA automatic composition machine. Then, the usual deprotection was performed, DNA was collected, and it refined in the high performance chromatography, and used for the following experiments.
Array number: The single stranded DNA of DNA **** by the 15 'HS-(CH2)6-O-PO2-O-ACTGGCCGTCGTTTTACA3' (4) BJ printer and the joint above-mentioned array number 1 to a substrate was dissolved in TE solution (10mM Tris-HCl (pH 8) / 1mM EDTA solution) so that the last concentration might become [ml] in about 400mg /, and the single stranded DNA solution was prepared (exact concentration is computed from absorption intensity).
[0090] Glycerol 7.5wt%, urea 7.5wt%, the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% shown by the thiodiglycol 7.5wt% and above-mentioned general formula (I) was prepared, and in addition to the above-mentioned DNA solution, it adjusted so that the last concentration of a single stranded DNA might be set to 8microM. The surface tension of this liquid was within the limits of 30 - 50 dyne/cm, and viscosity was 1.8cps (E type viscometer : Tokyo Keiki [Co., Ltd.] Co., Ltd. make). The ink tank for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) was filled up with this liquid, and the bubble jet head was equipped with it. In addition, the bubble-jet printer (tradename : BJC620; Canon [, Inc.], Inc. make) used here converts so that printing to a plate may be possible. Moreover, this bubble-jet printer is printable in the resolution of 360x720dpi. Subsequently, this printer was equipped with the glass plate processed above (2), and the liquid containing a probe nucleic acid was spotted on the glass plate. The distance of liquid ****** of a bubble jet head and the liquid adhesion side of a glass plate was 1.2-1.5mm here. Moreover, spotting performed 2 times of ****** in the direction of 360dpi after one spotting, and conditioning was carried out in the direction of 720dpi so that 5 times of ****** might be performed after one spotting. The glass plate was put into the humidification chamber for 30 minutes after the spotting end, and the maleimide machine on the front face of a glass plate and the thiol group of a nucleic-acid probe end were made to react. In addition, the discharge quantity of the

DNA probe solution per 1 discharging of the above-mentioned printer was about 24 pl(s).
[0091] (5) It is 1M after a reaction end with a blocking reaction maleimide machine and a thiol group, and about a glass plate. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the liquid containing DNA on the front face of a glass plate was flushed completely. Subsequently, the glass plate was dipped into 2% bovine-serum-albumin solution, it was left for 2 hours, and the blocking reaction was performed.
[0092] (6) DNA of the hybridization reaction array number 1 and the single stranded DNA which has a complementary base sequence were compounded with the DNA automatic composition machine, and the single stranded DNA which was made to combine a rhodamine with a five prime end, and labeled was obtained. It is 1M about this labeling single stranded DNA. It dissolved so that it might become the last concentration M of 1micro at NaCl / 50mM phosphate buffer solution (pH 7.0), and the probe array which was obtained above (5) in this solution and which carried out blocking processing was immersed, and the hybridization reaction was performed at the room temperature (25 degrees C) for 3 hours. Then, it is 1M about a probe array. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the probe nucleic acid and the single stranded DNA which was not hybridized were flushed. Next, the fixed quantity was carried out using the done type fluorescence microscope equipped with the filter set which connects image-analysis equipment (tradename : ARGUS 50; Hamamatsu Photonics make), and fits Rhodamine B in the amount of fluorescence of the spot of this probe array of a handstand.
[0093] (7) At the spot of the nucleic-acid probe of the array number 1 which are a resulting-indicator-ized single stranded DNA and a full match, it was the amount of fluorescence of 4600. Moreover, the probe array in the state where each spot after hybridization is carrying out firefly luminescence was observed using the fluorescence microscope (NIKON [CORP.] CORP. make). the probe array which starts this example as a result — a — between that it is in within the limits the diameter of whose each spot is almost circular and is about 70-100 micrometers, and the spots of which b contiguity is done — the diameter of each spot, and abbreviation — there is about 100-micrometer equal space and it became clear that each spot has been independent clearly mutually and that the row and column of c spot has gathered
[0094] This is very effective when making automatic detection of the spot hybridized on the probe array etc. perform.
[0095] Example 2 (manufacture of the nucleic-acid probe array using the bubble-jet printer, and detection of the target-nucleus acid using the probe array)
(1) The glass plate which performed surface treatment for probe arrays completely like (1) of the above-mentioned example 1 and (2) was prepared.
[0096] (2) The single strand nucleic acid of the array numbers 1-4 was compounded using the synthetic DNA automatic composition machine of probe DNA. In addition, the array number 2 and the thing which carried out 3 base change were made into the array number 3, and what was changed six bases was made into the array number 4 for what carried out 1 base change on the basis of the array number 1 which used the single strand nucleic acid of the array numbers 1-4 in the example 1. Moreover, the thiol (SH) machine was introduced by using Thiol-Modifier (product made from GlenResearch) for the single stranded DNA end of the array numbers 1-4 at the time of composition with a DNA automatic composition machine. Then, the usual deprotection was performed, DNA was collected, and it refined in the high performance chromatography, and used for the following experiments. The array of the array numbers 2-4 is shown below.
Array number : 25'HS- 6-O-PO2-O-ACTGGCCGTTGTTTTACA3' array number : 35'HS-(CH2)6-O-PO2-O-ACTGGCCGCTTTTTTACA3 (CH2) 'array number : **** of the DNA probe by the 45 'HS-(CH2)6-O-PO2-O-ACTGGCATCTTGTTTACA3' (3) BJ printer, And four kinds of ****** liquids are prepared using the single stranded DNA of the joint above-mentioned array numbers 1-4 to a substrate by the method indicated to (4) of the above-mentioned example 1, and the same method. Four ink tanks for bubble-jet printers used in the example 1 were filled up with each liquid, and the bubble jet head was equipped with each ink tank. Subsequently, this printer was equipped with the glass plate created by the same method as the above (1), and each of four sorts of nucleic-acid probes was spotted on this glass plate at 3x3mm each of four area of this glass plate. In addition, the pattern of spotting in each area presupposed that it is the same as that of an example 1. The glass plate was put into the humidification chamber for 30 minutes after the spotting end, and the maleimide machine and the thiol group were made to react.
[0097] (4) It is 1M after a reaction end with a blocking reaction maleimide machine and a thiol group, and about a glass plate. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the DNA solution on the front face of a glass plate was flushed completely. Subsequently, the glass plate was dipped into 2%

bovine-serum-albumin solution, it was left for 2 hours, and the blocking reaction was performed.
[0098] (5) DNA of the hybridization reaction array number 1 and the single stranded DNA which has a complementary base sequence were compounded with the DNA automatic composition machine, the rhodamine was combined with the five prime end, and the labeling single stranded DNA was obtained. It is 1M about this labeling single stranded DNA. It dissolved so that it might become the last concentration M of 1micro at NaCl / 50mM phosphate buffer solution (pH 7.0), and the probe array and hybridization reaction which were obtained by (4) were performed for 3 hours. Then, it is 1M about a probe array. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the probe nucleic acid and the single stranded DNA which was not hybridized were flushed. Next, each spot of this probe array was observed with the fluorescence microscope (NIKON [CORP.] CORP. make), and the fixed quantity was carried out using the done type fluorescence microscope equipped with the filter set which connects image-analysis equipment (tradename : ARGUS 50; Hamamatsu Photonics make), and fits Rhodamine B in the amount of fluorescence of a handstand.
[0099] (6) The amount of fluorescence of 2800 was obtained at the spot of the DNA probe of the array number 2 which has the mismatch array of one base to being the amount of fluorescence of 4600 at the spot of the DNA probe of the array number 1 which are a resulting-indicator-ized single stranded DNA and a full match. Moreover, at the spot of the DNA probe of the array number 3 which has 3 base mismatch, only the amount of fluorescence below the half of 2100 and a full match was obtained, and fluorescence was not observed in DNA of the array number 4 of 6 base mismatch. From the above thing, the single stranded DNA of a perfect complementarity was specifically detectable on the DNA array substrate.
[0100] Example 3 (the concentration and the bubble jet regurgitation property of the DNA probe in a liquid)
(1) The single stranded DNA which has the array of the array number 5 shown below in composition of a DNA probe was compounded using the DNA automatic composition machine, it was dissolved in TE solution (10mM Tris-HCl (pH 8) / 1mM EDTA solution) so that concentration might become [ml] respectively in about 0.2mg [ml] /, 2mg [ml] /, and 15mg /, and three kinds of DNA probe solutions with which concentration differs were prepared (exact concentration was computed from absorption intensity).
Array number : Regurgitation glycerol 7.5% by the 55'GCCTGATCAGGC3'(2) BJ printer, The solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1% which has the structure shown by the above-mentioned general formula (I) is prepared 7.5% of ureas, and thiodiglycol 7.5%. In addition to the probe solution with a concentration of 0.2mg [/ml] adjusted above (1), the last concentration diluted [ml] this solution in about 0.02mg (3microM) /. The ink tank for bubble-jet printers which used this liquid in the above-mentioned example 1 was filled up, and the head of the bubble-jet printer which used this ink tank in the example 1 was equipped.
[0101] Next, this printer was equipped with the aluminum board of A4 size, and it spotted to the area of the 3x5 square inches of this aluminum board. Spotting here was set up so that the above-mentioned area might spot by the density of 360x720dpi. Moreover, the ink of marketing [to the beginning] for BJ620 as control was printed on this aluminum board. This operation was performed to a total of four aluminum boards.
[0102] Next, the nucleic-acid probes by which the spot was carried out on each aluminum board were collected using TE solution, it refined by gel filtration technique and the amount of the refined recovery nucleic-acid probe was measured with the absorption spectrum. The amount of recoveries of the nucleic-acid probe called for theoretically here is as follows. That is, the volume per [which is breathed out from the head of the printer used for this example] drop is a 24pico liter. And since there are four aluminum boards which spotted in the area of 3x5 square inches by the density of 360x720dpi, it is set to 24(pico liter) x(720x360) x (3x5) x four-sheet =373microl. The absorbance in 260nm which the probe nucleic acid of this amount shows, and the absorbance in 260nm of the collected nucleic-acid probe are shown in drawing 3 .
[0103] In the completely same operation as the above (2), 15mg /followed each probe solution ml the concentration of 2mg/ml. In addition, the last concentration of the nucleic-acid probe of each ****** liquid was set to 30microM (0.2mg/(ml)) and 225microM (1.5mg/(ml)). The result of the absorbance which the absorbance and the amount of probe nucleic acids calculated theoretically which the probe nucleic acid collected from each solution shows show is shown in drawing 3 .
[0104] (3) It was a value near the value with which the discharge quantity with an actual nucleic-acid probe is theoretically expected that result drawing 3 shows. In the regurgitation of the nucleic-acid probe using a bubble jet process, quantitative loss of the nucleic-acid probe by the bad debt of the nucleic-acid probe to the heater section of a bubble jet head etc. is not accepted from this thing. Moreover, the spotting in process

to the aluminum board using the liquid of each concentration and no trouble of a head, for example, the non-regurgitation etc., were generated. Moreover, when the spot of the ink for bubble-jet printers and the spot of a nucleic-acid probe which spotted to the aluminum board as control were contrasted visually, the spotting situation of the spot created using the liquid of concentration 3microM and 30microM was almost the same as that of it of an ink spot. Moreover, as for the spot created using the liquid with a concentration [M] of 225micro, some disorder was accepted as compared with the ink spot.

[0105] Example 4 (examination of the influence which a bubble jet process has on a nucleic-acid probe)

(1) It prepared so that base length 10mer (synthetic compounds), oligoA (40- 60mer; Pharmacia manufacture) and poly (dA) which consist of a synthetic adenine (it is henceforth indicated as "A") of a nucleic-acid probe, and a (300-400mer; Pharmacia manufacture) might be diluted with TE solution, respectively and the last concentration might become [ml] in 1mg /, and the nucleic-acid probe solution with which length differs was prepared. In addition, the base sequence (array number : 6) of 10mer(s) is as follows.

Array number: Regurgitation glycerol 7.5wt% of the DNA solution by the 65" [AAAAAAAAAA3] (2) bubble-jet printer, the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals) 1wt% shown by the urea 7.5wt% and above-mentioned general formula (I) was prepared, and each nucleic-acid probe solution created above (1) in this solution was diluted so that the last concentration might become in ml and about 0.1mg /.

[0106] Each nucleic-acid probe solution with which the cartridge was filled up like the example 3 was made to breathe out on an aluminum board, and the spotting situation was observed visually. As a result about the nucleic-acid probe of base length 10mer and 40-60mer, the probe array with which the spot which became independent on the aluminum board was tidily located in a line was obtained. Moreover, although the same probe array was fundamentally obtained also about the nucleic-acid probe of 300-400mer, the portion with which adjoining spots are connected was accepted. Physical-properties change of the liquid with which the base chain of a nucleic-acid probe originates in a ******** arises, and this is considered because the directivity of the regurgitation from a bubble jet head became incorrectness a little.

[0107] Next, the spots on the probe array created using each nucleic-acid probe solution were collected like the example 3. 100micro of collected nucleic-acid probe solutions l was analyzed by Antiphase HPLC, and comparison with the solution in front of the regurgitation investigated the existence of cutting of a nucleic-acid probe. In addition, 7 - 70% acetonitrile concentration gradient containing 1M triethylamine acetate performed elution of Antiphase HPLC. Consequently, a DNA fragment which is considered to have been cut was not observed but, therefore, it has checked that the nucleic-acid probe had not received transformation by the regurgitation in a bubble jet process, either. Moreover, as a result of performing the fixed quantity of the collected nucleic-acid probe like an example 3, as shown in drawing 4 , as for the nucleic-acid probe of three kinds of length, the amounts as a theoretical value were collected mostly.

[0108] In (4) of example 5 (examination of reaction time) example 1, except having carried out room temperature (25 degrees C) neglect of the surface treatment glass plate which spotted the nucleic-acid probe into the humidification chamber overnight for 10 minutes and 90 minutes, the probe array was manufactured like the example 1 and the hybridization reaction was presented with each probe array. About the probe array made to react as a result 90 minutes and overnight, fluorescence intensity of the same grade as the fluorescence intensity which the probe array altogether obtained in the example 1 shows was given. It became clear to have ended mostly the ligation reaction of the maleimide machine on the front face of a glass plate and the thiol group of a nucleic-acid probe end in 30 minutes from this. On the other hand, the reaction time of the probe array for 10 minutes was about 70% of the amount of fluorescence compared with it of an example 1.

[0109] Example 6 (manufacture of the PNA probe array using the bubble-jet printer, and detection of the target-nucleus acid using the probe array)

(1) The glass plate which performed surface treatment for probe arrays completely like (1) of the above-mentioned example 1 and (2) was prepared.

[0110] (2) The protein nucleic acid (PNA) (product made from Japanese par SEPUTIBU) which has the base sequence of the synthetic following array numbers 7 and 8 of Probe PNA was prepared. A cysteine residue (Cys and notation) is combined with an amino terminus (equivalent to the five prime end of DNA), and, as for this PNA, the thiol group is introduced into the amino terminus as the result. Moreover, the PNA probe of the array number 8 little salt-machine-changes the PNA probe of the array number 7.

Array number : 7 NCys-NH 2-O- (CH2) 2-O-CH2 CONH-ACTGGCCGTCGTTTTACAC array number : The

regurgitation of the PNA probe according to a 2-O-(CH2)2-O-CH2 CONH-ACTGGCCGTTGTTTACAC(3) BJ printer 8 NCys-NH (CH2), (CH2) And the PNA probe of each joint above to a substrate is dissolved so that the last concentration may be set to 80microM at the 0.1wt% trifluoroacetic acid of 100microl. Subsequently, glycerol 7.5wt% and urea 7.5wt% and thiodiglycol 7.5wt%, and the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% shown by the above-mentioned general formula (I) — the trifluoroacetic-acid solution of Above PNA — in addition, it adjusted so that the last concentration of a PNA probe might be set to 8microM The surface tension of this liquid was within the limits of 30 - 50 dyn/cm, and viscosity was within the limits of 1-5cps.
[0111] It spotted in each area on the glass plate which created these PNA probe solutions of each above (1) the same with having indicated to (3) of an example 2. It put after a spotting end and into the 3-hour humidification chamber, and the maleimide machine and the thiol group were made to react.
[0112] In addition, the discharge quantity of the PNA probe solution per 1 discharging of the above-mentioned printer was about 24 pl(s).
[0113] (4) It is 1M after a reaction end with a blocking reaction maleimide machine and a thiol group, and about a glass plate. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the liquid containing PNA on the front face of a glass plate was flushed completely. Subsequently, the glass plate was dipped into 2% bovine-serum-albumin solution, it was left for 3 hours, and the blocking reaction was performed.
[0114] (5) The single stranded DNA which has PNA of the hybridization reaction array number 7 and a complementary base sequence was compounded with the DNA automatic composition machine, and the single stranded DNA which was made to combine a rhodamine with a five prime end, and labeled was obtained. This labeling single stranded DNA was dissolved so that it might become 10mM phosphate buffer solution (pH 7.0) with last concentration 5nM (the amount of solutions of 1ml), the PNA probe array which was obtained above (4) in this DNA solution and which carried out blocking processing was immersed, and the hybridization reaction was performed at the room temperature (25 degrees C) for 12 hours. Then, 10mM phosphate buffer solution (pH 7.0) solution washed the probe array, and the PNA probe and the single stranded DNA which was not hybridized were flushed. Next, the fixed quantity was carried out using the done type fluorescence microscope equipped with the filter set which connects image-analysis equipment (tradename : ARGUS 50; Hamamatsu Photonics make), and fits Rhodamine B in the amount of fluorescence of the spot of this probe array of a handstand.
[0115] (6) In the PNA probe of the array number 8 which has 1 base mismatch array to having been the amount of fluorescence of 2400 in the PNA probe of the array number 7 which are a resulting-indicator-ized single stranded DNA and a full match, it was 1100 of an abbreviation half. The single stranded DNA of a perfect complementarity was specifically detectable on the PNA array from the above thing.
[0116] Moreover, the probe array in the state where each spot after hybridization is carrying out firefly luminescence was observed using the fluorescence microscope (NIKON [CORP.] CORP. make). the probe array which starts this example as a result — a — about 50-micrometer space is between that it is in within the limits the diameter of whose each spot is almost circular and is about 200 micrometers, and the spot of which b contiguity is done, and it became clear that each spot has been independent clearly mutually and that the row and column of c spot has gathered
[0117] This is very effective when making automatic detection of the spot hybridized on the probe array etc. perform.
[0118] Furthermore, since there was no need of making hybridization reaction time and the solution used for removal of a subsequent unreacted single stranded DNA containing a sodium chloride, it did not need to be cautious of a deposit of a sodium chloride during observation of fluorescence, and the hybrid on a probe array was able to be detected more easily. Moreover, a preservation top did not have the need for seal, either and was easy handling.
[0119] In addition, although a larger reason than the spot of the probe array which the diameter of a spot of a PNA probe obtained in the example 1 is not clear, as a result of having acquired knowledge that water solubility is inferior in a PNA probe a little as compared with a DNA probe and both water-soluble difference making the surface tension of each ink-jet ****** produce a difference, as for this invention persons, it is guessed that the diameter of a spot is a different thing.
[0120] Example 7 (manufacture and its evaluation of the glass substrate with a black matrix for probe arrays which introduced the epoxy group into the front face)
(1) The glass substrate (50mmx50mm) which consists of synthetic quartz was cleaned ultrasonically using

2wt% sodium-hydroxide solution, subsequently UV ozonization was performed, and the front face was defecated. the silane coupling agent (tradename : KBM403; Shin-Etsu Chemical Co., Ltd. make) containing the silane compound (gamma-glycidoxypropyltrimetoxysilane) which combined the epoxy group — 1wt% — the 50wt% methanol solution to contain was stirred under the room temperature for 3 hours, and the methoxy machine in the above-mentioned silane compound was hydrolyzed Subsequently, this solution was applied to the above-mentioned substrate front face by the spin coater, it heated and dried for 5 minutes at 100 degrees C, and the epoxy group was introduced into the substrate front face.
(2) The DEEP-UV resist (negative resist for black matrices) (tradename : BK- 739P; the NIPPON STEEL chemistry incorporated company make) which next contains carbon black is applied so that the thickness after hardening may be set to 5 micrometers by the spin coater, and with the hot plate, at 80 degrees C, this substrate was heated for 5 minutes and stiffened. Pro squeak tee exposure was carried out using the mask by which patterning was carried out so that the distance between the contiguity wells in drawing 5 (X) might serve as 100 micrometers and a square whose configuration of a well is 100micrometerx100micrometer to a 1cmx1cm field using a DEEP-UV aligner, subsequently, negatives were developed with the developer of inorganic alkali solution using the spin developing machine, pure water washed further, and the developer was removed completely. Next, it dried simply using the spin dryer, it heats for 30 minutes at 180 degrees C in clean oven after that, and actual hardening of the resist was carried out, 2500 wells have been arranged in the predetermined array and the adjoining well obtained the substrate isolated by the black matrix. In addition, the capacity of each well is calculated with a 50pico liter (pl). Due to this time, the contact angle to the water of a black matrix front face cannot get wet easily with 93 degrees, and the contact angle to the water at the base of a well tended to get wet with 35 degrees.
[0121] (3) The ink tank for bubble-jet printers (tradename : BJC620 : Canon [, Inc.], Inc. make) was filled up with the Rhodamine B solution of 10microM, and the bubble jet head of the bubble-jet printer used in the aforementioned example 1 was equipped. and the printer was equipped with the solid phase prepared by the above (1) and (2), and Rhodamine B solution was supplied to the check pattern (even — setting) at the well of this solid phase In addition, the amount of supply per one well is about 50 pl(s). Moreover, the **** positioning accuracy of this printer is **2.5 micrometers. Next, another ink tank was filled up with the solution of 10microM amino [FITC], the bubble jet head of the above-mentioned printer was equipped, and another well which adjoins the well which supplied Rhodamine B solution previously was supplied. It is water-soluble to have used Rhodamine B and amino one FITC here, and it is because **** from an ink-jet head being performed easily, and the state and cross contamination of a liquid which were supplied to the well by observation of fluorescence can be checked.
[0122] (4) The fluorescence microscope (NIKON [CORP.] CORP. make) was equipped with G excitation filter (for Rhodamine B), and B excitation filter (for amino FITC), and the state of each solution supplied to the well by one 100 times the scale factor of this was observed by fluorescence. As a result, each solution was uniformly supplied in the well, without forming a drop. Moreover, mutually from each well, the fluorescence of other coloring matter was not observed and cross contamination was not accepted.
[0123] Example 8 (manufacture of the probe array using the substrate of an example 7, and detection of the target-nucleus acid using it)
(1) The substrate with a black matrix (BM) was created by the same method as an example 7.
(2) two nucleotides prepared [one nucleotide] the probe (array number : 11) (all — the Nippon Flour Mills Co., Ltd. make and HPLC grade) of a mismatch to the probe (array number : 10) of a mismatch, and the oligomer of the array number 9 to the oligomer (array number : 9) of 18 **** which combined the amino group with the hydroxyl group of a five prime end through the phosphoric-acid machine and the hexamethylene as a DNA probe, and the oligomer of the array number 9 The base sequence of the oligomer of the array number 9 is an array complementary to some base sequences of the multiple cloning site of M13mp18-ssDNA which is a single stranded DNA. the following — array number: — the base sequence of 9-11 and the structure of linkage are shown
array number: — 95 — 'NH2-(CH2)6-O-PO2-O-TGTAAAACGACGGCCAGT3' — array number: — 105 — 'NH2-(CH2)6-O-PO2-O-TGTAAAACCACGGCCAGT3' — array number: — 115 — 'NH2-(CH2)6-O-PO2-O-TGTATAACCACGCCCAGT3' — the DNA probe of the (3) above-mentioned array numbers 9-11 — receiving — completeness — the complementary single stranded DNA was compounded Next, each DNA probe and the single stranded DNA were dissolved in TE solution (pH 8) which contains NaCl by the concentration of 50mM (s) so that the last concentration might be set to 100microM, and the DNA probe solution and the single

stranded DNA solution were prepared. And the solution which contains a complementary single stranded DNA in each DNA probe to 100micro of solutions I containing a DNA probe was 100microl Added, it mixed, each mixed solution was linearly cooled over 2 hours from 90 degrees C to 25 degrees C, and the hybrid of each DNA probe and each single strand nucleic acid was made to form. Next, the above-mentioned array number: In addition to the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% shown by the thiodiglycol 7.5wt% and aforementioned general formula (I) glycerol 7.5wt% and urea 7.5wt% in the solution containing the hybrid of each DNA probe of 9-11, it adjusted so that the hybrid last concentration might be set to 8microM. Each surface tension of these liquids containing the hybrid of each DNA probe was within the limits of 30 - 50 dyne/cm, and viscosity was also within the limits of 1-5cps (E type viscometer : Tokyo Keiki [Co., Ltd.] Co., Ltd. make).

[0124] Next, three ink tanks for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) were prepared, each ink tank was filled up with three sorts of above-mentioned hybrid solutions, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the glass substrate with BM created by the above (1) and (2) was set, and the solution which contains the hybrid of the DNA probe of the array number 9 first was supplied to the well (131 of drawing 6) of eye one train. Next, the solution containing the hybrid of the DNA probe of the array number 10 was supplied to 2 **** well (133 of drawing 6) which adjoins the well of eye the one above-mentioned train, and the solution which contains the hybrid of the DNA probe of the array number 11 further was supplied to the well (135 of drawing 6) of eye three trains adjoin the well of eye the two above-mentioned train. In addition, to one well, which hybrid solution was breathed out 4 times and about 100 pl supply was carried out. Although the supplied hybrid solution rose from opening of a well and it existed when each well was observed under the microscope, although this amount was twice [about] the capacity of one well, it has stopped in the well by the hydrophobic matrix, and the cross contamination between wells was not observed.

[0125] next, a substrate — the constant temperature of 25 degrees C and 100% of humidity — the amino group of every 12 hours and a probe and the epoxy group of a well were made to react to a constant humidity chamber In addition, since the amino group of the base of a probe forms a complementary perfect single stranded DNA and a perfect complementary hybrid, it does not react with the epoxy group of each well.

[0126] (4) Next, 80-degree C pure water washed the substrate for 10 minutes, and while making the complementary strand which has constructed the probe combined with the substrate, and the hybrid dissociate from a probe, it washed away. Subsequently, the substrate was processed under the room temperature in ethanolamine solution 1% for 1 hour, and ring breakage of the unreacted epoxy group in each well was carried out. Next, pure water washed the substrate and it dried.

[0127] Since ring breakage of the DNA probe in a well and the epoxy group which did not react is carried out, and it turns into a hydroxyl group by **** of the above (4) and a hydroxyl group exists also in the ethanolamine made to react, a hydrophilic property becomes high more and the base of a well becomes advantageous in the case of supply to the well of the solution containing the below-mentioned target single stranded DNA.

[0128] (5) The single stranded DNA of a perfect complementarity to the DNA probe of the array number 9 was dissolved in TE solution (pH 8) which next contains NaCl by the concentration of 50mM(s) so that the last concentration might be set to 10microM, it was immersed, the probe array which introduced the epoxy group into the well obtained above (4) in this solution was lowered over 2 hours from 80 degrees C to 25 degrees C, and the high buri tie ZESHON reaction was performed. Subsequently, after TE buffer solution (pH 8) which contains NaCl of 10mM(s) at 20 degrees C washed the substrate for 20 minutes, the spin **** machine removed the surface penetrant remover.

[0129] (6) The 2-methyl -4 and 6-screw (4-N and N-dimethylamino phenyl) pyrylium eye OTAIDO (it abbreviates to "P2" below) which emit fluorescence only after intercalate in a double strand nucleic acid were dissolved in TE solution (pH 8.0) which next contains NaCl by the concentration of 50mM(s) so that the concentration might be set to 10microM, the ink tank for the above-mentioned ink jet printers was filled up with this solution, and it was attached in the head of the above-mentioned ink jet printer. Moreover, the substrate which performed hybridization above (5) is set to the above-mentioned printer. After supplying 100 pls of P2 solutions at a time to each well, in order to prevent dryness, it is left for 5 minutes within the exclusive chamber of 100% of humidity. having held in a chamber — a done-a handstand type microscope (tradename: — IMT2; Olympus optical incorporated company make —) Scale factor: 100 times, the filter cube for fluorescence microscopes (595nm (transparency) from filter 455nm for excitation) An ICCD camera

(tradename : C2400- 87; Hamamatsu Photonics make) and an image processor (tradename : ARGUS 50; Hamamatsu Photonics make) are connected to use for dichroic mirror 620nm and barrier filter 610nm for fluorescence to 725nm (transparency). The observation fixed quantity of the fluorescence was carried out. In addition, observation area is 25micrometerx25micrometer, integration x64, and ARGUS. The amplification level of 50 was set up suitably.
[0130] Consequently, from the well which combined the DNA probe of the array number 11, the almost same fluorescence intensity of 1200-1500 as the background was observed. The fluorescence intensity of 3500-3900 was observed from the well which the fluorescence intensity of 9800-10300 is observed [well] from the well which, on the other hand, combined the DNA probe of the array number 9, and combined the DNA probe of the array number 10. Furthermore, when each solid phase is washed for 10 minutes at 35 degrees C using TE buffer solution and fluorescence intensity is measured again, from the well which combined the DNA probe of the array number 10, only fluorescence intensity of the same grade as the background is observed.
[0131] These results showed that a hybridization reaction could be performed in each well and the array number 9 and a perfect complementary target-nucleus acid could be further detected specifically by using the probe array concerning this example.
[0132] Example 9 (alternative supply of the reacting matter to each well of the probe array of an example 8, and reaction with a probe)
(1) The substrate which combined the DNA probe of the array numbers 9-11 like the example 8 was prepared.

[0133] (2) Three kinds of perfect complementary single stranded DNAs were compounded to the DNA probe of the array numbers 9-11. The three above-mentioned kinds of single stranded DNAs were dissolved in TE solution (pH 8) which contains NaCl by the concentration of 50mM(s) so that each concentration might be set to 100microM. Three ink tanks for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) were prepared, each ink tank was filled up with three sorts of above-mentioned single stranded DNA solutions, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the substrate prepared above (1) was also set to the printer, and supplied every 100 pls per well of solutions which contain a perfect complementary single stranded DNA respectively to the well which the DNA probe of the array numbers 9-11 has combined. When the state of each well was observed under the microscope at this time, it turns out that the solution of the matter which bleeding of liquid and cross contamination are not observed and should be made to react to each well of a probe array individually can be supplied.
[0134] (3) After making a hybridization reaction perform in each well like an example 8 next, P2 solution was supplied to each well like the example 8, and the hybrid was detected by observing fluorescence. Consequently, the fluorescence of the intensity of 9800-10300 was observed from all wells. Supplied the reacting matter to each well of a solid phase probe array individually from this, the probe and the reacting matter were made to react in each well, and it was checked that an object is detectable as a result of a reaction.
[0135] Example 10 (hydrophilicity-ized processing at the base of a well of the substrate of an example 7)
(1) The glass substrate which has a black matrix pattern like an example 7 was prepared.
[0136] (2) UV ozonization was performed on the near front face in which the black matrix of this substrate is formed. At this time, the contact angle to the water of a black matrix front face was in 93 degrees and the state of being hard to get wet, and the contact angle to the water at the base of a well was 22 degrees, and was in the state of being easy to get wet as compared with it at the base of a well of the substrate with a black matrix obtained in the example 7. This is considered to be an effect by the above-mentioned UV ozonization.
[0137] (3) When the supply situation of the ink-jet regurgitation liquid to a well was observed using Rhodamine B and solution amino [FITC] like the example 7 next, each solution of both was uniformly supplied in the well, without forming a drop within a well. Unlike the case where the solid phase which does not have a well on a front face in using the solid phase which equipped the front face with the well as solid phase of a probe array and which has a flat and uniform surface characteristic is used, it is not necessary to stop ink-jet regurgitation liquid in the position limited as much as possible, and becomes more advantageous to detection of the reaction of the probe and target matter with which making it go and continue fully performs ink-jet regurgitation liquid on a well base behind rather. The hydrophilicity-ized processing at the base of a well indicated to this example is a method desirable as the one embodiment. Moreover, it turns out that the ink-jet process was used for each coloring matter solution, and it has supplied mutually from the well to which each

coloring matter is supplied at each well, without having not observed other coloring matter but producing cross contamination.
[0138] Example 11 (the process of the probe array using the solid phase which supplied and obtained the liquid for the functional-group introduction for probe fixation by the ink-jet method to each well of BM formation substrate, and its use)
(1) The substrate equipped with the black matrix like the example 7 was prepared.
[0139] (2) the silane coupling agent (tradename : KBM603; Shin-Etsu Chemical Co., Ltd. make) containing the silane compound (N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane) which combined the amino group — 1wt% — the 10wt% methanol solution to contain was stirred under the room temperature for 3 hours, and the methoxy machine in the above-mentioned silane compound was hydrolyzed Subsequently, the ink tank for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) was filled up with this solution, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the substrate prepared above (1) was also set to the printer, and supplied the silane-coupling-agent solution containing the silane compound with which the methoxy machine was understood an added water part to the well. [as well as an example 8] this substrate — the constant temperature of 25 degrees C and 100% of humidity — after leaving it in a constant humidity chamber for 30 minutes, with pure water, spin dryness was washed and carried out, after that, and the amino group was introduced into the base of each well [100 degrees C] [for 30 minutes]

[0140] (3) next, the last concentration becomes a 5wt%DMSO solution with 5wt(s)% about SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate (Aldrich make) — as — dissolving — this solution — the above (2) — the same — carrying out — an ink jet printer — each well — every 100 pls — supplying — subsequently — the constant temperature of 30 degrees C and 100% of humidity — the substrate was left in the constant humidity chamber for 2 hours Next, it is pure, a substrate is washed, spin dryness was carried out, and the maleimide machine was introduced into the base of each well.
[0141] (4) two nucleotides prepared [one nucleotide] the probe (array number : 14) (all — the Nippon Flour Mills Co., Ltd. make and HPLC grade) of a mismatch to the probe (array number : 13) of a mismatch, and the oligomer of the array number 12 to the oligomer (array number : 12) of 18 **** which combined the thiol group with the hydroxyl group of a five prime end through the phosphoric-acid machine and the hexamethylene as a DNA probe, and the oligomer of the array number 12 the following — array number: — the base sequence of 12-14 and the structure of linkage are shown
Array number : 125'HS- 6-O-PO2-O-TGTAAAACGACGGCCAGT3' array number : 135'HS-(CH2)6-O-PO2-O-TGTAAAACCACGGCCAGT (CH2) 3' array number: Each DNA probe of the above-mentioned array numbers 12-14 was dissolved in the phosphate buffer solution of 145'HS-(CH2)6-O-PO2-O-TGTATAACCACGCCCAGT3'(5)10mM so that the last concentration might be set to 10microM. The well of the substrate which created each DNA probe solution above (3) like the above-mentioned example 8 was supplied. When each well was observed under the microscope, although the supplied DNA probe solution rises and exists from opening of a well, it has stopped in the well by the hydrophobic matrix, and cross contamination was not observed. this substrate — the constant temperature of 30 degrees C and 100% of humidity — pure water performed washing and spin dryness to the constant humidity chamber ****** and after that for 2 hours, the thiol group of each DNA probe was made to react with the maleimide machine of each well, and the DNA probe was combined with the substrate
[0142] (6) the DNA probe of the array number 12 — receiving — completeness — TE solution which compounds a complementary single stranded DNA and contains NaCl by the concentration of 50mM(s) — this single stranded DNA — last — a wave — it dissolved so that a degree might be set to 10microM It was immersed, the DNA probe joint substrate obtained above (5) in this solution was lowered over 2 hours to 80 degrees C - 25 degrees C, and hybridization was performed. Next, after washing a substrate for 20 minutes at 20 degrees C using TE solution (pH 8) which contains NaCl by the concentration of 10mM(s), the spin dryer removed the penetrant remover on the front face of a substrate.
[0143] (7) YOYO-1 which is the reagent which emits fluorescence only after intercalates in a hybrid was dissolved so that the last concentration might become TE solution included by concentration 50mM with 10microM about NaCl (pH 8). It supplied 100 pls of this solution at a time to each well of a substrate which processed the above (6) using the ink jet printer like the above (2), and the observation fixed quantity of the fluorescence was carried out like the example 8 (B excitation filter is used). In addition, the signal amplification level of Argus50 is the same as that of an example 8.

[0144] Consequently, from the well which combined the DNA probe of the array number 14, the almost same fluorescence intensity of 1800-2000 as the background was observed. The fluorescence intensity of 3100-3300 was observed from the well which the fluorescence intensity of 7500-8000 is observed [well] from the well which, on the other hand, combined the DNA probe of the array number 12, and combined the DNA probe of the array number 13. Furthermore, when solid phase is washed for 10 minutes at 35 degrees C using TE buffer solution and fluorescence intensity is measured again, from the well which combined the DNA probe of the array number 13, only fluorescence intensity of the same grade as the background is observed.
[0145] These results showed that a hybridization reaction could be performed in each well and the array number 9 and a perfect complementary target-nucleus acid could be further detected specifically by using the probe array concerning this example.
[0146] The substrate which combined the DNA probe of the array numbers 12-14 like the example 12 (1) example 11 was prepared.
[0147] (2) Three kinds of perfect complementary single stranded DNAs were compounded to the DNA probe of the array numbers 12-14. The three above-mentioned kinds of single stranded DNAs were dissolved in TE solution which contains NaCl by the concentration of 50mM(s) so that each concentration might be set to 10microM. In addition, pH of each single stranded DNA solution is 8. Three ink tanks for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) were prepared, each ink tank was filled up with three sorts of above-mentioned single stranded DNA solutions, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the substrate prepared above (1) was also set to the printer, and supplied every 100 pls per well of solutions which contain a perfect complementary single stranded DNA respectively to the well which the DNA probe of the array numbers 12-14 has combined. When the state of each well was observed under the microscope at this time, it turns out that the solution of the matter which bleeding of liquid and cross contamination are not observed and should be made to react to each well of a probe array individually can be supplied.
[0148] (3) After making a hybridization reaction perform in each well like an example 11 next, YOYO-1 solution was supplied to each well like the example 11, and the hybrid was detected by observing fluorescence. Consequently, the fluorescence of the intensity of 7500-8000 was observed from all wells. Supplied the reacting matter to each well of a solid phase probe array individually from this, the probe and the reacting matter were made to react in each well, and it was checked that an object is detectable as a result of a reaction.
[0149] Example 13 (process of the probe array using the substrate which was flooded with the solution for epoxy-group introduction in BM formation substrate, and introduced the epoxy group into the well)
(1) The substrate with a black matrix was created according to the publication of an example 7 of (2).
[0150] (2) According to the publication of an example 7 of (1), the 1wt% solution of the silane coupling agent (tradename : KBM403; Shin-Etsu Chemical Co., Ltd. make) containing the silane compound (gamma-glycidoxypropyltrimetoxysilane) which combined the epoxy group was stirred under the room temperature for 1 hour, and the methoxy machine in the molecule of this silane compound was hydrolyzed. Subsequently, the solid phase prepared above (1) into this solution was immersed for 30 minutes under the room temperature, pure water washed this solid phase after that, and water was removed by the nitrogen gas style, and the epoxy group was introduced into the well base. [120 degrees C] [for 5 minutes] At this time, there was a contact angle to the water on the front face of BM in the state of being hard to get wet with 95 degrees, and the contact angle to the water of a well pars basilaris ossis occipitalis was in 33 degrees and the state of being easy to get wet. Thus, introduction of the epoxy group at the base of a well is possible also by processing the solid phase after BM formation by the silane coupling agent.
[0151] (3) The DNA probe of array number:9-11 was combined with the base of a well according to the method indicated to (3) of the above-mentioned example 8, and (4).
[0152] (4) The single stranded DNA which has a complementary base sequence to the array number 9 was compounded by DNA automatic composition Quercus acutissima, and the labeling single stranded DNA which combined the tetramethyl rhodamine with the five prime end through the hexanol amine linker was obtained. This labeling single stranded DNA was dissolved so that the last concentration might be set to 2microM in NaCl at TE solution (pH 8) included by the concentration of 50mM(s). It was immersed, the DNA probe joint substrate obtained above (3) in this solution was lowered over 2 hours from 80 degrees C to 25 degrees C, and the hybridization reaction was performed. They are 10mM(s) about a probe array after that. It washed for 20 minutes at 29 degrees C using the NaCl/TE buffer solution (pH 8), and the probe nucleic acid and the

single stranded DNA which was not hybridized were flushed. Next, the fixed quantity of the amount of fluorescence from each well was carried out like the example 8.
[0153] (5) From the well which combined the DNA probe of the array number 9 which are a resulting-indicator-ized single stranded DNA and a full match, the amount of fluorescence of 8500-9400 was checked. Moreover, from the well which the amount of fluorescence of 2800-3400 is observed [well] from the well which combined the DNA probe of the array number 10, and combined the DNA probe of the array number 11 again, only the about 1200 to 1500 amount of fluorescence was observed. Moreover, when the above-mentioned probe array was washed for 10 minutes at 35 more degrees C using 10 mMNaCl/TE buffer solution (pH 8), the amount of fluorescence from the well which combined the DNA probe of the array number 10 fell even to the level of the background. Therefore, even if it uses the probe array concerning the example of ******, it turns out that specific detection of the hybrid target matter is possible.
[0154]
[Effect of the Invention] it can spot without [as explained above, without it gives a damage to this probe for the spot which contains a probe on solid phase by using ink-jet technology according to this invention, and] producing a satellite spot Moreover, the quality probe array equipped with the probe spot mutually independently and with high density can be efficiently manufactured by using this method.
[0155] Furthermore, it can judge more correctly and quickly whether the target matter exists in a sample by being able to acquire [rather than] many information for the probe array which can be inspected to accuracy even from a small amount of sample about the target matter according to this invention, and using it. The structure of the target matter in a sample can be specified more correctly and quickly by using this probe array similarly.
[0156] Moreover, according to this invention, a matrix pattern can be formed in a front face as solid phase of a probe array, and the position gap of some of supplies of the probe solution to solid phase or supplies of the sample to solid phase can also be coped with by using the solid phase which prepared the well. Moreover, much more highly precise-ization of detection of the target matter, specification of structure, etc. was attained by making a matrix support various functions.
[0157]
[Layout Table] Array number : The length of 1 array : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end A thiol group Junction sequence ACTGGCCGTCGTTTTACA array number : The length of 2 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : a five prime end — a thiol group — junction sequence ACTGGCCGTTGTTTTACA array number: — length [of three arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array synthetic DNA array ACTGGCCGCTTTTTTACA array number: — length [of 4 arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array synthetic DNA array ACTGGCATCTTGTTTACA array number: — length [of five arrays]: — mold [of 12 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Synthetic DNA array GCCTGATCAGGC array number : length [of 6 arrays]: — mold [of ten arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array synthetic DNA array AAAAAAAAAA array number: — length [of seven arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides Composition PNA : At the N' end A cysteine residue Junction sequence ACTGGCCGTCGTTTTACA array number : The length of 8 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides Composition PNA : At the N' end A cysteine residue Junction sequence ACTGGCCGTTGTTTTACA array number : The length of 9 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end The amino group Junction sequence TGTAAAACGACGGCCAGT array number : The length of 10 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end The amino group Junction sequence TGTAAAACCACGGCCAGT array number : The length of 11 arrays : Mold of 18 arrays : number [of nucleic-

acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end The amino group Junction sequence TGTATAACCACGCCCAGT array number : The length of 12 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end A thiol group Junction sequence TGTAAAACGACGGCCAGT array number : The length of 13 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end A thiol group junction sequence TGTAAAACCACGGCCAGT array number: — length [of 14 arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array information: besides a synthetic DNA — 5' end — a thiol group — junction sequence TGTATAACCACGCCCAGT

[Translation done.]

* NOTICES *

Japan Patent Office is not responsible for any
damages caused by the use of this translation.

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

TECHNICAL FIELD

[The technical field to which invention belongs] this invention relates to the method of spotting a probe to solid phase, a probe array, its manufacture method, the method of detection of the target single strand nucleic acid using the probe array, and the specification-ized method of the base sequence of a target single strand nucleic acid.

[Translation done.]

* NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

PRIOR ART

[Description of the Prior Art] Use of the matter which can be specifically combined for example, with this target-nucleus acid as one of the technology which can perform quickly and correctly determination of the base sequence of a nucleic acid, detection of the target-nucleus acid in a sample, and identification of various bacteria, and the probe array which arranged many so-called probes in on solid phase is proposed.
[0003] the method of compounding the nucleic-acid probe on (1) solid phase as the general manufacture method of such a probe array, as indicated by the European Patent No. 373203 official report (EP 0373203B1), for example, and ** (2) — the method of supplying the nucleic-acid probe compounded beforehand on solid phase etc. is learned As advanced technology with which the detail of the above-mentioned method of (1) is indicated, for example, a U.S. Pat. No. 5405783 official report (USP5405783) is mentioned.
[0004] Moreover, the method of arranging cDNA in in the shape of an array, using micro pipetting as the method of the above (2) is indicated by a U.S. Pat. No. 5601980 official report (USP5601980), "a science (Science)", the 270th volume, 467 pages, and (1995), for example.
[0005] By the way, since the method of the above (1) is making the direct nucleic-acid probe compound on solid phase, it does not need to compound a nucleic-acid probe beforehand. However, it is difficult to refine the probe nucleic acid compounded on solid phase. It depends on the precision of the base sequence of a nucleic-acid probe for precision, such as sequencing of the nucleobase using the probe array, and detection of the target-nucleus acid in a sample, greatly. Therefore, improvement in the precision of a nucleic-acid probe is just going to ask for the further improvement as a process of a probe array with the more nearly quality method of the above (1).
[0006] On the other hand, the method of the above (2) can refine a nucleic-acid probe in advance of the combination to solid phase, while the synthetic step of a nucleic-acid probe is needed in advance of fixation in the solid phase of a nucleic-acid probe. In a present stage, it is considered by this reason as a process of a more nearly quality probe array for the method of the above (2) to be more desirable than the method of the above (1).
[0007] However, the technical problem of the method of the above (2) is in the method of spotting a nucleic-acid probe with high density to solid phase. For example, when performing base sequence determination of a nucleic acid using a probe array, it is desirable to arrange various nucleic-acid probes on solid phase as much as possible. Moreover, when detecting variation of a gene efficiently, it is desirable to arrange the nucleic-acid probe which has an array corresponding to each variation on solid phase. Furthermore, as for extraction of blood etc., in detection of the target-nucleus acid in a sample, and the variation of a gene and detection of a deficit, it is specifically desirable extraction of the sample from a subject and to stop to small quantity as much as possible, and it is desirable that the information on the base sequence of many in a small amount of sample as possible can therefore be acquired. When it thinks from these points, it is desirable that 10000 or more nucleic-acid probes are arranged at the probe array for example, at the 1 inch angle.

[Translation done.]

* NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

EFFECT OF THE INVENTION

[Effect of the Invention] it can spot without [as explained above, without it gives a damage to this probe for the spot which contains a probe on solid phase by using ink-jet technology according to this invention, and] producing a satellite spot Moreover, the quality probe array equipped with the probe spot mutually independently and with high density can be efficiently manufactured by using this method.
[0155] Furthermore, it can judge more correctly and quickly whether the target matter exists in a sample by being able to acquire [rather than] many information for the probe array which can be inspected to accuracy even from a small amount of sample about the target matter according to this invention, and using it. The structure of the target matter in a sample can be specified more correctly and quickly by using this probe array similarly.
[0156] Moreover, according to this invention, a matrix pattern can be formed in a front face as solid phase of a probe array, and the position gap of some of supplies of the probe solution to solid phase or supplies of the sample to solid phase can also be coped with by using the solid phase which prepared the well. Moreover, much more highly precise-ization of detection of the target matter, specification of structure, etc. was attained by making a matrix support various functions.
[0157]
[Layout Table] array number: — length [of one array]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array . Information besides a synthetic DNA : To a five prime end A thiol group Junction sequence ACTGGCCGTCGTTTTACA array number : The length of 2 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : a five prime end — a thiol group — junction sequence ACTGGCCGTTGTTTTACA array number: — length [of three arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array synthetic DNA array ACTGGCCGCTTTTTTACA array number: — length [of 4 arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array synthetic DNA array ACTGGCATCTTGTTTACA array number: — length [of five arrays]: — mold [of 12 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Synthetic DNA array GCCTGATCAGGC array number : length [of 6 arrays]: — mold [of ten arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array synthetic DNA array AAAAAAAAAA array number: — length [of seven arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides Composition PNA : At the N' end A cysteine residue Junction sequence ACTGGCCGTCGTTTTACA array number : The length of 8 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides Composition PNA : At the N' end A cysteine residue Junction sequence ACTGGCCGTTGTTTTACA array number : The length of 9 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end The amino group Junction sequence TGTAAAACGACGGCCAGT array number : The length of 10 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end The amino group Junction sequence TGTAAAACCACGGCCAGT array number : The length of 11 arrays : Mold of 18 arrays : number [of nucleic-

acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end The amino group Junction sequence TGTATAACCACGCCCAGT array number : The length of 12 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end A thiol group Junction sequence TGTAAAACGACGGCCAGT array number : The length of 13 arrays : Mold of 18 arrays : number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array Information besides a synthetic DNA : To a five prime end A thiol group junction sequence TGTAAAACCACGGCCAGT array number: — length [of 14 arrays]: — mold [of 18 arrays]: — number [of nucleic-acid chains]: — single strand topology: — nucleic acid besides kind: of a straight chain-like array information: besides a synthetic DNA — 5' end — a thiol group — junction sequence TGTATAACCACGCCCAGT

[Translation done.]

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] As a result of examining many things under such a situation, this invention persons find out that a probe can be spotted very with high density using ink-jet **** technology, and came to succeed in this invention.
[0009] And the purpose of this invention is efficiently extremely to offer the method of spotting correctly on solid phase, without doing damage for the probe of a minute amount at this probe.
[0010] Moreover, other purposes of this invention are [rather than] about many information about a nucleic acid even from a small amount of sample to offer the probe array which can be inspected to accuracy.
[0011] Moreover, the purpose of further others of this invention is to offer the method of manufacturing efficiently, without damaging a probe for the probe array which the probe has combined on solid phase.
[0012] Furthermore, other purposes of this invention are to offer the method of detecting efficiently the target matter which may be contained in the sample.
[0013] Furthermore, other purposes of this invention are again to offer the specification-ized method of the structure of the target matter where the information about the structure of the target matter can be acquired even from a small amount of sample.

[Translation done.]

* NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

MEANS

[Means for Solving the Problem] The spotting method which takes like 1 operative condition as for this invention that the above-mentioned purpose can be attained is characterized by having the process which the liquid which contains a combinable probe specifically to the target matter is supplied [process] to a solid phase front face by the ink-jet method, and makes it adhere to this solid phase front face.
[0015] By using the spotting method concerning the above-mentioned mode, a probe can be given correctly and efficiently on solid phase, and a probe array can be manufactured efficiently.
[0016] Moreover, the probe array which takes like 1 operative condition as for this invention is characterized by having the spot of the probe which has been independent mutually to two or more parts on the front face of solid phase by the density of 10000 or more pieces in a 1 square inch. According to the probe array concerning the above-mentioned mode, many information can be acquired even from a small amount of sample from having the spot very with high density.
[0017] Moreover, the manufacture method of the probe array which takes like 1 operative condition as for this invention is the manufacture method of the probe array which has the spot which contains a combinable probe independently specifically to the target matter in two or more parts on the front face of solid phase, and is characterized by having the process which makes the liquid containing this probe supply and adhere to the position on this front face of solid phase using the ink-jet method. The probe array by which the spot has been arranged with high density can be manufactured efficiently, without harming a probe according to this mode.
[0018] Moreover, the method of detection of the target matter which takes like 1 operative condition as for this invention which can attain the above-mentioned purpose Each spot and this sample of the probe array which has the probe specifically combined to the target matter which may be contained in the sample as two or more spots which became independent mutually on solid phase are contacted. In the method of detecting a reactant with this target matter and a probe, and detecting the existence of this target matter in this sample on this solid phase Each of this spot is characterized by being formed by spotting the liquid containing this probe on solid phase by the ink-jet method. According to this mode, the target matter is efficiently detectable.
[0019] Furthermore, the specification-ized method of the structure of the target matter which takes like 1 operative condition as for this invention that the above-mentioned purpose can be attained The process which prepares the probe array which is the method of specifying the structure of the target matter contained in a sample, and was equipped with the spot of the probe specifically combined with a solid phase front face to this specific matter; The process which detects combination with process; which contacts this sample at this spot and this target matter, and this probe, It is characterized by ****(ing). When it is a single strand nucleic acid even from a small amount of sample by using this mode, the structure of the target matter, for example, the target matter, in this sample, the base sequence can be specified efficiently.
[0020] In addition, it is recognized if it is not appropriate for a USP No. 5601980 official report to use conventional ink-jet technology for spotting of a nucleic-acid probe. that is, the contamination of the spots of the nucleic-acid probe with which it is indicated that use of the ink jet printer technology of making little ink breathing out by the pressure wave (pressure wave) is not appropriate, the pressure wave for the ink regurgitation causes a temperature rise with rapid ink temperature as the reason, and there is possibility do an injury to a nucleic-acid probe, and spilling of the ink at the time of the regurgitation adjoins is caused in - of 31st line the 52nd line of the 2nd column of a USP No. 5601980 official report — danger The drop of the liquid which contains a nucleic-acid probe at the nose of cam of a micropipette in a USP No. 5601980 official

report using gas pressure on it is made to form, acting as the monitor of the size of this drop, when predetermined size is reached, pressure impression is stopped, and the method of supplying this drop on solid phase and manufacturing a probe array is indicated.

[0021] Moreover, the method of determining the base sequence of a target-nucleus acid as a USP No. 5474796 official report using forming the matrix of a hydrophobic property and a hydrophilic property in a solid phase front face, using piezo electric impulse jet pump equipment (Piezoelectric Impulse Jet Pump Apparatus) for the hydrophilic portion for four kinds of bases, being able to breathe out one by one, and manufacturing an oligonucleotide array in total and it is indicated.

[0022] However, these advanced technology is made to breathe out the nucleic-acid probe which has the base sequence of predetermined length beforehand using ink-jet technology, and it is not indicated at all about the technology of making a nucleic-acid probe arrange with high density and correctly.

[0023]

[Embodiments of the Invention] (Probe array process outline) Drawing 1 and drawing 2 are outline explanatory drawings of the manufacture method of of the probe array concerning this invention, for example, a nucleic-acid probe array. The liquid supply system (nozzle) which holds the liquid with which 101 contains in drawing 1 , the probe, for example, the nucleic-acid probe, as regurgitation liquid, possible [the regurgitation], the solid phase (for example, transparent glass plate etc.) with which, as for 103, this nucleic-acid probe should be combined, and 105 are bubble jet heads equipped with the mechanism in which this liquid is made to give and breathe out heat energy which are kinds of an ink-jet head. 104 is a liquid containing the nucleic-acid probe breathed out from the bubble jet head 105. Moreover, drawing 2 is the A-A line cross section of the bubble jet head 105 of drawing 1 , and the liquid with which 105 contains a bubble jet head and the nucleic-acid probe with which 107 should be breathed out in drawing 2 , and 117 are substrate portions which have the exoergic section which gives regurgitation energy to this liquid. The substrate portion 117 contains the base material 116 currently formed with the good alumina of the exoergic resistor layer 113 currently formed with the electrode 111-1,111-2 currently formed with the protective coat 109 currently formed by the silicon oxide etc., aluminum, etc., Nichrome, etc., the accumulation layer 115, and thermolysis nature etc.

[0024] The liquid 107 containing a nucleic-acid probe is coming to the regurgitation orifice (delivery) 119, and forms the meniscus 121 with the predetermined pressure. If an electrical signal joins an electrode 111-1,111-2 here, the field (foaming field) shown by 123 generates heat rapidly, a foam will be generated into the liquid 107 which has touched here, a meniscus will breathe out by the pressure, a liquid 107 will breathe out from an orifice 119, and it will fly toward the front face of solid phase 103. Although the amount of the liquid in which the regurgitation is possible changes with sizes of the nozzle etc. using a bubble jet head equipped with such structure, it is possible to control, for example to about 4-50 pico l., and it is very effective as a means to arrange a nucleic-acid probe with high density.

(Relation between regurgitation liquid and solid phase)

(Spot diameter on solid phase) In order to make it a value (it is a 1x106 individual grade as 10000 or more pieces and an upper limit for example, to 1 inch **) which described above the density on the solid phase of a nucleic-acid probe, as for the diameter of a spot of each nucleic-acid probe, it is desirable that the spot with it has been independent mutually. [that desirable and it is about 20-100 micrometers and] [mutual] And such a spot is determined by the property of the liquid breathed out from a bubble jet head, the surface characteristic of the solid phase to which this liquid adheres, etc.

[0025] (Property of regurgitation liquid) This liquid that a bubble jet head to the regurgitation was possible, and was breathed out from the head as a liquid for regurgitation reaches the position of the request on solid phase, and any liquids can be used if this nucleic-acid probe does not receive an injury further at the time of the mixed state with a nucleic-acid probe, and the regurgitation.

[0026] If it considers as the property of this liquid from a viewpoint of the regurgitation nature from a bubble jet head, 30 or more dyn/cm have [the viscosity] 1-15cps and surface tension desirable [and]. Moreover, when viscosity is made to 1-5cps and surface tension is made into 30 - 50 dyn/cm, the impact position on solid phase will become very exact, and it will be used especially suitably.

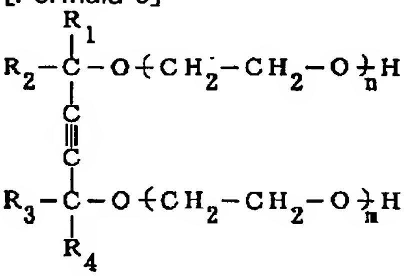
[0027] next — if the stability of the nucleic-acid probe at the time among the ink-jet regurgitation property of this liquid and a liquid of the bubble jet regurgitation is taken into consideration — the inside of a liquid — for example, 2mer(s)- it is especially desirable 0.05-500microM and to make the nucleic-acid probe of 2mer-60mer contain by the concentration of 2-50microM especially 5000 mer

[0028] (Regurgitation liquid composition) As composition of the liquid breathed out from a bubble jet head It is

what does not affect it substantially to a nucleic-acid probe as described above when it mixes with a nucleic-acid probe, and when it is made to breathe out from a bubble jet head. And if the conditions that the liquid composition in which the regurgitation is possible is desirable are normally fulfilled to solid phase using a bubble jet head Especially the liquid that contains the acetylene alcohol shown, for example by a glycerol, a urea, the thiodiglycol or ethylene glycol, isopropyl alcohol, and the following formula (I) although not limited is desirable.

[0029]

[Formula 5]



(I)

[0030] (R1, R2, R3, and R4 express the shape of a straight chain and the branched-chain alkyl group of carbon numbers 1-4 to an alkyl group and a concrete target among the above-mentioned formula (I), and m and n express an integer respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)

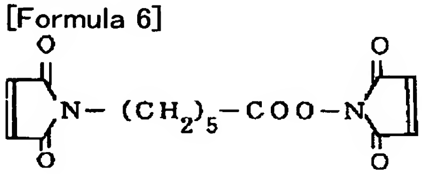
Furthermore, the liquid which contains 0.5 - 1wt% more preferably is suitably used 0.02 - 5wt% in the acetylene alcohol which a glycerol is shown by 5 - 10wt% 5 - 10wt%, and is specifically shown [urea] by the 5 - 10wt% and above-mentioned formula (I) in a thiodiglycol.

[0031] The configuration of the spot at the time of making the liquid which contains a nucleic-acid probe from a bubble jet head breathe out, and making it adhere on solid phase, when this liquid is used is circular, and when the breathed-out range does not spread and a nucleic-acid probe is spotted with high density, connection at the adjoining spot can be suppressed effectively. Furthermore, transformation of the nucleic-acid probe which it spotted on solid phase is not accepted, either. In addition, the property of the liquid used for manufacture of the nucleic-acid probe array of this invention is not limited to the above-mentioned thing. For example, when structure like a well which prevents mixing between the spots which the liquid given on solid phase with the bubble jet head spreads on this solid phase, and are adjoined is prepared in a solid phase front face, even if the viscosity and surface tension of a liquid, and also the base length and concentration of the above of a nucleic-acid probe are also out of range, it can use.

[0032] (Solid phase and kind of functional group of a nucleic acid) The position limited further is made to stop the spot of the nucleic-acid probe made to adhere on solid phase, and the method of making the both sides of a nucleic-acid probe and solid phase combine mutually the functional group in which a reaction is possible as a means by which it is effective in order to prevent contamination with the adjoining spot more certainly, and it is effective in combining a nucleic-acid probe firmly on solid phase is mentioned.

[0033] (A sulfhydryl group and maleimide machine) The example using the combination of for example, a maleimide machine and a thiol (-SH) machine as a desirable example is given. That is, a thiol (-SH) machine is combined with the end of a nucleic-acid probe, by processing so that a solid phase front face may have a maleimide machine, the thiol group of a nucleic-acid probe and the maleimide machine on the front face of solid phase which were supplied to the solid phase front face can react, a nucleic-acid probe can be fixed, and, as a result, the spot of a nucleic-acid probe can be formed in the position on solid phase. When the thing which the liquid of the composition which described above the nucleic-acid probe which has a thiol group was made to dissolve especially in an end is given to the solid phase front face which introduced the maleimide machine using the bubble jet head, a nucleic-acid probe solution forms a very small spot on solid phase. Consequently, a spot with a small nucleic-acid probe can be formed in the position on the front face of solid phase. In this case, the well which consists of a hydrophilic property and a hydrophobic matrix is formed in a solid phase front face, and the need of preparing beforehand composition which prevents connection between spots is not accepted.

[0034] For example, the liquid adjusted so that it might become within the limits which the viscosity which contains the nucleic-acid probe of base length 18mer by the concentration M of 8micro, and surface tension

described above a bubble-jet printer (tradename: -- BJC620; Canon [, Inc.], Inc. make --) However, the interval of the nozzle of solid phase and a bubble jet head is set as about 1.2-1.5mm using what was converted possible [printing] monotonously. When it is made to breathe out from this nozzle (discharge quantity is an about 24pico liter), on solid phase, a spot with a diameter of about about 70-100 micrometers can be formed. Moreover, visually, the spot (a "satellite spot" is called henceforth) originating in a droplet when a liquid reaches a solid phase front face was not accepted at all. Although the reaction of the maleimide machine on this solid phase and the sulfhydryl group of a nucleic-acid probe end is based also on the conditions of the liquid breathed out, it is completed in about 30 minutes under a room temperature (25 degrees C). In addition, this time is short as compared with the case where a piezo jet head is used for the regurgitation of a liquid. Although the reason is not clear, by the bubble jet process, it is thought that the temperature of the liquid which contains the nucleic-acid probe in a head by the principle rises, the reaction efficiency of a maleimide machine and a thiol group goes up as a result, and reaction time is shortened.
[0035] In addition, when using the combination of a maleimide machine and a thiol group, it is desirable to make the solution containing a nucleic-acid probe contain a thiodiglycol. Under neutrality and weak alkaline conditions, a thiol group forms a disulfide bond (-S-S-) and has a bird clapper in a dimer. However, a reactant fall with the thiol group and maleimide machine by dimer formation can be prevented by adding a thiodiglycol.
[0036] Although various methods can be used as the introductory method of the maleimide machine on the front face of solid phase, it is possible by, making an amino silane coupling agent react to a glass substrate for example, and making the reagent (EMCS reagent : product made from Dojin) containing N-(6-maleimide KAPURO yloxy) SUKUSHI imide (N-(6-Maleimidocaproyloxy) succinimide) shown with the amino group and following structure expression below react.
[0037]
[Formula 6]


[0038] Moreover, in case the nucleic-acid probe which the thiol group combined compounds DNA automatically for example, using a DNA automatic composition machine, it can be compounded by using 5'-Thiol-ModifierC6 (product made from Glen Research) as a reagent of a five prime end, and it is obtained by refining by the high performance chromatography after the usual deprotection reaction.
[0039] (The amino group and epoxy group) As combination of the functional group used for fixation, the combination of an epoxy group (on solid phase) and the amino group (nucleic-acid probe end) etc. is mentioned besides the combination of the above-mentioned thiol group and the above-mentioned maleimide machine. Introduction of the epoxy group on the front face of solid phase applies the polyglycidylmethacrylate which has an epoxy group to the solid phase front face which consists of a resin, or the silane coupling agent which has an epoxy group is applied to a glass solid phase front face, and glass, the method of making it react, etc. are mentioned.
[0040] As described above, when a functional group which reacts to a solid phase front face and the end of a single strand nucleic-acid probe mutually, and forms covalent bond is introduced, this nucleic-acid probe and solid phase are combined more firmly. Moreover, since a bonding site with the solid phase of this nucleic-acid probe can always be used as an end, the state of the nucleic-acid probe in each spot can be made uniform. As a result, the conditions of the hybridization of the nucleic-acid probe and target-nucleus acid in each spot will gather, and it is thought that the detection of a target-nucleus acid and the specification of a base sequence which improved further are attained. Furthermore, carrying out covalent bond of the nucleic-acid probe which the functional group attached to the end, and the solid phase can produce a probe array quantitatively compared with the nucleic-acid probe fixed on solid phase by noncovalent bonds (for example, electrostatic combination etc.), without producing the difference of the amount of combination of the probe DNA by the difference in an array or length. Furthermore, since all the base sequence portions of a nucleic acid contribute to a hybridization reaction again, the efficiency of a hybridization reaction can be raised remarkably. Moreover, you may introduce an about one to seven-carbon number alkylene machine as a linker portion between the functional groups which participate in the reaction of the portion and solid phase which participate in hybridization with the target-nucleus acid of a single strand nucleic-acid probe. When combining

a nucleic-acid probe with solid phase by this, a predetermined distance can be established between this solid phase front face and this nucleic-acid probe, and much more improvement in the reaction efficiency of a nucleic-acid probe and a target-nucleus acid can be expected.

[0041] (Process of an array) One of the most desirable modes in the present condition of the manufacture method of the probe array which next starts this invention is explained. A liquid including acetylene alcoholic (for example, tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% of the structure shown by the above-mentioned general formula (I) is prepared thiodiglycol 7.5wt% urea 7.5wt% glycerol 7.5wt% as a liquid which distributes a nucleic-acid probe first. Next, the single strand nucleic-acid probe which the thiol group has combined with the end and about 2-5000 mers of whose length are about 2-60 mers especially, for example is compounded using a DNA automatic composition machine. subsequently, this nucleic-acid probe — in 0.05-500microM and the range which is 2-50microM especially, 1-15cps of concentration is mixed so that the viscosity of this liquid may be set to 1-5cps and 30 or more dyn/cm of surface tension may set it the above-mentioned liquid with 30 - 50 dyn/cm especially especially, and it considers as the liquid for regurgitation And it is filled up with this liquid for regurgitation in the nozzle of for example, a bubble jet head. Moreover, according to the above-mentioned method, the maleimide machine is introduced into solid phase on the front face. And the distance of the field and the nozzle side of a bubble jet head where the maleimide machine of this solid phase has combined this solid phase and this bubble jet head makes about 1.2-1.5mm approach, makes this bubble jet head drive, and makes this liquid breathe out. It is desirable to set it as a printing pattern which the spot on solid phase does not connect as regurgitation conditions here. For example, when it spots on the conditions of making the empty regurgitation carry out in the direction of 360dpi twice [after / the 1 time regurgitation], and making the empty regurgitation carry out in the direction of 720dpi after / the 1 time regurgitation / 5 times, when the resolution of the bubble jet head used for spotting is 360x720dpi, it is possible for the space between each spots to be set to about 100 micrometers, and to fully prevent contamination with the adjoining spot.

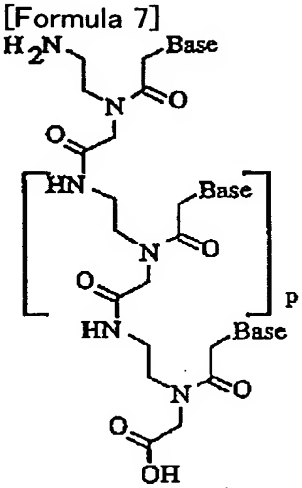
[0042] Subsequently, the reaction of the maleimide machine on solid phase and the thiol group of the nucleic-acid probe in a liquid progresses, and this solid phase is put for example, into a humidification chamber until this nucleic-acid probe is certainly fixed to solid phase. As described above, a room temperature (about 25 degrees C) is enough as this time at about 30 minutes. It is on solid phase after that, an unreacted nucleic-acid probe is flushed, and a nucleic-acid probe array is obtained.

[0043] After fixing this nucleic-acid probe to a solid phase front face here for the purpose of aiming at improvement in the detection precision in the case of performing detection of a target-nucleus acid etc. (S/N ratio), using this nucleic-acid probe array, it is desirable to block so that it may not combine with the target-nucleus acid with which the nucleic-acid probe uncombined portion of this solid phase is contained in a sample. Blocking is possible by dipping for example, this solid phase into 2% bovine-serum-albumin solution for about 2 hours, for example, or making the maleimide machine which has not been combined with the nucleic-acid probe on the front face of solid phase disassemble. For example, it is possible even if it uses DTT (dithiothreitol), beta-mercaptoethanol, etc. However, bovine-serum-albumin solution is most suitable, considering the effect which prevents adsorption of Indicator DNA. In addition, that what is necessary is just to carry out if needed, the process of this blocking performs supply to this probe array of a sample in limitation to each spot, and when there is no adhesion of the sample to parts other than a spot substantially, it does not need to perform it. Moreover, a well is beforehand formed in solid phase, and the process of blocking can be skipped when portions other than the well are processed so that a nucleic-acid probe cannot adhere easily.

[0044] Thus, you may constitute the probe array to produce so that it may have two or more spots which may constitute so that it may have two or more spots containing the same nucleic-acid probe, corresponding to the use, and contain respectively a nucleic-acid probe of a different kind. And the probe array by which the nucleic-acid probe has been arranged with high density by such method is used for detection of a target single strand nucleic acid, specification of a base sequence, etc. after that. for example, when the base sequence which may be contained in the sample uses for detection of a known target single strand nucleic acid The single strand nucleic acid which has a complementary base sequence to the base sequence of this target single strand nucleic acid is used as a probe. The probe array by which two or more spots containing this probe are arranged on solid phase is prepared. After putting on the bottom of a condition which supplies a sample to each spot of this probe array, and this target single strand nucleic acid and a nucleic-acid probe hybridize at it, the existence of the hybrid in each spot is detected by known methods, such as fluorescence

detection. The existence of the target matter in a sample is detectable with it. Moreover, in using for specification of the base sequence of the target single strand nucleic acid contained in the sample, two or more candidates of the base sequence of this target single strand nucleic acid are set up, and it spots to this solid phase by using as a probe the single strand nucleic acid which has a complementary base sequence respectively to this base sequence group. Subsequently, after putting on the bottom of a condition which supplies a sample to each spot and this target single strand nucleic acid and a nucleic-acid probe hybridize, the existence of the hybrid in each spot is detected by known methods, such as fluorescence detection. Thereby, the base sequence of a target single strand nucleic acid can be specified. Moreover, application to screening of the chemical which has the property combined with screening and DNA of the specific base sequence which DNA binding protein recognizes, for example as other uses of the probe array concerning this invention can be considered.
[0045] (Kind of ink-jet head) In the still more nearly above-mentioned explanation, although only the composition which performs grant to the solid phase of a nucleic-acid probe with a bubble jet head was explained, it is also possible to use the piezo jet head on which the liquid in a nozzle can be breathed out in this invention using ****** of a piezo-electric element, and it closes. However, since the ligation reaction to solid phase is completed for a short time and the secondary structure of DNA is also canceled by heat when a bubble jet head is used, as described above, a bubble jet head is an ink-jet head used more suitable for this invention at the point that the efficiency of the hybridization reaction following a degree can also be raised.
[0046] You may form two or more spots on solid phase simultaneously using the ink-jet head equipped with two or more heads so that the nucleic-acid probes contained between further two or more spots might differ.

(PNA/DNA) this invention was explained so far, using a nucleic-acid probe as an example of a probe. As an example of a nucleic-acid probe, a deoxyribonucleic-acid (DNA) probe, a ribonucleic-acid (RNA) probe, and a peptide nucleic-acid (PNA) probe are included. PNA is an synthetic oligonucleotide which has the structure where four sorts of bases (an adenine, a guanine, a thymine, cytosine) contained in DNA combine with the peptide principal chain instead of a sugar-phosphate ester principal chain, and are shown in the following formula (II).
[0047]
[Formula 7]



(II)

[0048] ("Base" shows among a formula any of four kinds of bases (an adenine, a cytosine, a thymine, guanine) which constitute DNA they are.) Moreover, p expresses the base length of PNA.
PNA is compoundable by the method learned as for example, a tBOC type solid phase synthesis method or a Fmoc type solid phase synthesis method. and strong resistance [as opposed to enzymes, such as a nuclease and a protease, as compared with the oligonucleotide of nature / PNA /, such as DNA and RNA,] — having — the inside of a blood serum — enzyme-cleavage — almost — or it happens at all and is stable Moreover, since it has neither the sugar part nor the phosphoric-acid machine, it is not necessary to adjust salt concentration at the time of hardly being influenced of the ionic strength of a solution, therefore making PNA and a target single strand nucleic acid react etc., and in order that there may be no still more nearly electrostatic rebounding, it is thought that the way of the hybrid of PNA and a target single strand nucleic

acid is excellent in thermal stability as compared with the hybrid of a DNA probe and a target single strand nucleic acid or the hybrid of an RNA probe and a target single strand nucleic acid. And it is promising as a probe used for detection of a target-nucleus acid, or the determination of a base sequence from these properties. And the manufacture method of the nucleic-acid probe array concerning said this invention is effective when a PNA probe is applied as a nucleic-acid probe, and the PNA probe array by which the PNA probe has been arranged with high density and with high precision can be manufactured easily. Like a DNA probe or an RNA probe as a method of making the position where it was limited on solid phase specifically stopping a PNA probe, and attaining densification of a probe array Being able to use the method of introducing into each on the end of a PNA probe, and the front face of solid phase the functional group which has reactivity mutually, one of the desirable combination of a reactant basis is the combination of the thiol group (PNA end) same with having mentioned above, and a maleimide machine (solid phase front face). Introduction of the thiol group to a PNA end is attained by introducing the cysteine (CH(NH2) (COOH) CH2SH) machine which contains a thiol group in the amino terminus (equivalent to the five prime end of DNA) of for example, a PNA probe. Introduction of the cysteine to the amino terminus of a PNA probe can be performed by making the amino group of the amino terminus of for example, a PNA probe, and the carboxyl group of a cysteine react. Moreover, a cysteine can also be combined with the amino terminus of a PNA probe through a linker by making the amino group of the amino terminus of a PNA probe, and the carboxyl group of a suitable linker which has an amino group and the carboxyl group like for example, N2H(CH2)2O (CH2)2OCH2COOH react, and subsequently making the amino group of this linker, and the carboxyl group of a cysteine react. Thus, when a joint machine with solid phase is introduced through a linker, only a predetermined distance can make a reactive site with the target matter of a PNA probe estrange from solid phase, and much more improvement in hybridization efficiency is expected.

[0049] Moreover, in case PNA has a low case for some of the base length as compared with DNA of the base length with the same solubility over water and the liquid for ink-jet regurgitation is prepared, after dissolving PNA in trifluoroacetic acids (for example, 0.1wt% trifluoroacetic-acid solution etc.) etc. beforehand, it is desirable to prepare in the property which suits the ink-jet regurgitation using said various solvents. It is desirable to make it dissolve especially in a trifluoroacetic acid, when preventing the denaturation to the cystine by oxidization of the thiol group in the cysteine residue of a PNA end and aiming at much more improvement in the reaction efficiency of the thiol group of PNA, and the maleimide machine on the front face of solid phase. Moreover, although the reaction time of the thiol group introduced into the end of a DNA probe or an RNA probe and the maleimide machine on the front face of solid phase is enough in 30 minutes as described above (when a bubble jet head is used), even if it is the case where a bubble jet head is used in PNA, it is desirable to make it react for about 2 hours.

[0050] Furthermore, as a probe, it is not limited to a nucleic-acid probe, but the oligopeptide or polypeptide which has a combinable receptor and a specific amino acid sequence, a combinable oligopeptide and a combinable polypeptide, protein (for example, an antibody, an antigen, an enzyme, etc.), etc. can be used as a probe as specifically as a combinable ligand and a combinable ligand as specifically as the target matter in the sample used as the candidate for detection / analysis and the matter which can be combined specifically, for example, a receptor, In this case, the sulfhydryl group of the cysteine residue by which all are contained in protein can be used for a reaction.

[0051] According to the manufacture method of a probe array including the process which supplies a probe solution to solid phase using an ink-jet regurgitation process as explained above, a probe array can be formed very easily. When a functional group is introduced into each so that covalent bond may be especially formed between a nucleic-acid probe and a solid phase front face, the spots which adjoin even if it does not have a well etc. on a solid phase front face beforehand, namely, uses flat solid phase with a uniform surface characteristic (the ease of getting wet to water etc.) for it substantially do not connect. Moreover, the nucleic-acid probe array by which the nucleic-acid probe was arranged often [precision] and with high density as a result can be manufactured by the low cost very efficiently.

[0052] In addition, it does not eliminate at all that this uses the solid phase which equipped the front face with the well in this invention. For example, when the matrix pattern (a "black matrix" is called henceforth) of light impermeability nature is beforehand formed between the wells to which a probe solution is supplied, the detection precision (SN ratio) of a case so that hybridization of the probe on solid phase and the target matter may be detected optically (for example, detection of fluorescence) can be raised further. Moreover, though a position gap of some arises in supply to the well of a probe solution when a front face establishes

the low matrix of the compatibility over a probe solution between adjoining wells, a probe solution can be smoothly supplied to a desired well. You may use the solid phase which equipped the front face with the well for the purpose of using such an effect. The solid phase which has a matrix on a front face below, its manufacture method, and the operation in this embodiment of this solid phase are explained.
[0053] An example of the probe array in this mode is shown in drawing 5 . Drawing 5 (A) is a plan and drawing 5 (B) is the BB cross section. This probe array has the structure which formed the matrix pattern 125 which has the frame structure in which the crevice (well) 127 arranged in the shape of a matrix was formed on solid phase 103. The well 127 mutually isolated by the matrix 125 (heights) was formed as a breakthrough in a matrix pattern (Japanese common chestnut omission section), and the side attachment wall consists of heights, and is in the state where the front face of solid phase 103 was exposed to the base 129. The amount of [of solid phase 103] surface outcrop forms the front face in which a probe and combination are possible, and the probe (un-illustrating) is being fixed to the predetermined crevice.
[0054] When the method of measuring and detecting the fluorescence to which detection, for example, a reactant, emits the reactant of a probe and the target matter optically is used for it, and improvement in detection sensitivity, a S/N ratio, and reliability is taken into consideration, as for the material which forms a matrix pattern, what has shading nature is desirable. As such a material, metals (chromium, aluminum, gold, etc.), a black resin, etc. are mentioned, for example. As a resin of this black, the thing which made the black color and the black pigment contain is mentioned to resins, such as an acrylic, a polycarbonate, polystyrene, polyethylene, a polyimide, an acrylic-acid monomer, and urethane acrylate, and photosensitive resins, such as a photoresist. As an example of a photopolymer, UV resist, a DEEP-UV resist, ultraviolet-rays hardening resin, etc. can be used, for example. As a UV resist, positive resists, such as negative resists, such as an cyclization polyisoprene-aromatic screw azide system resist and a phenol resin-aromatic azide compound system resist, and a novolak-resin-diazo naphthoquinone system resist, can be mentioned.
[0055] as a DEEP-UV resist — as a positive resist — for example, a polymethylmethacrylate, a polymethylene sulfone, poly hexafluoro butyl methacrylate, the poly methyl isopropenyl ketone, and bromination — dissolution inhibitor system resists, such as radiolysis type polymer resists, such as a poly 1-trimethylsilyl propyne, and cholic-acid o-nitrobenzyl ester, etc. can be mentioned, and the polyvinyl phenol -3, a 3'-diazide diphenyl sulfone, polymethacrylic-acid glycidyl, etc. can be mentioned as a negative resist
[0056] As ultraviolet-rays hardening resin, the polyester acrylate which is chosen from oxime system compounds, such as a benzophenone and its substitution derivative, a benzoin and its substitution derivative, an acetophenone and its substitution derivative, and a benzyl, etc. and which contained one sort or two sorts or more of photopolymerization initiators about 2 to 10% of the weight, epoxy acrylate, urethane diacrylate, etc. can be mentioned. Carbon black and a black organic pigment can be used as a black pigment.
[0057] In addition, when not detecting the reactant of a probe and the target matter optically, or when the light from a matrix does not affect optical detection of a reactant, using the object of un-shading nature as a matrix pattern formation material is not barred at all.
[0058] Next, the method of carrying out the coat of the photoresist on the resin metallurgy group which carried out the coat to the substrate front face as one method of forming a matrix pattern using material which was described above, and carrying out patterning of the resin according to processes, such as etching, after patterning is mentioned. Moreover, if it is a photosensitive resin, it is also possible by hardening the resin itself exposure, development, and if needed according to the process of the photo lithography using the photo mask to carry out patterning.
[0059] When a matrix 125 is made into the product made of a resin here, the front face of a matrix 125 becomes hydrophobic. This composition is desirable when using the solution of a drainage system as a solution containing the probe supplied to a well. That is, though a probe solution is supplied with a position gap of some in case a probe solution is used for a well and the ink-jet method is supplied to it, a probe solution will be supplied to a desired well very smoothly. moreover — the case where the probe of a different kind is supplied between the wells which adjoin simultaneously — these wells — between different probe solutions supplied in between — being mixed (cross contamination) — protecting also becomes possible
[0060] Usually, since the probe solution of living body related substances, such as a peptide and a nucleic acid, is a solution of a drainage system in many cases, in such a case, a matrix pattern can use the composition of water repellence and a bird clapper suitably.
[0061] Next, how to consider the base (outcrop on the front face of solid phase) of a well as the composition in which a probe and combination are possible is explained. The functional group made to hold on the base of

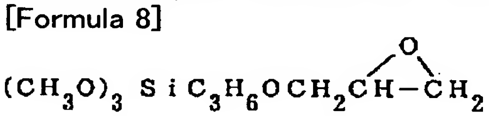
a well changes with combination with the functional group which a probe is made to support. For example, when using the nucleic-acid probe which introduced the thiol group into the end as a probe, the thiol group of the nucleic-acid probe supplied to the well by introducing a maleimide machine into a solid phase front face as mentioned above forms the maleimide machine and covalent bond on the front face of solid phase, and a nucleic-acid probe is fixed to a solid phase front face. To the nucleic-acid probe which has an amino group at the nucleic-acid probe end similarly, introduction of the epoxy group on the front face of solid phase is desirable. As other combination of such a functional group, introduction of the amino group on the front face of solid phase is desirable to the nucleic-acid probe which has a carboxyl group (based on the introduction to the nucleic-acid probe end of a succinimide derivative) at the end, for example. Although the fixing nature to the solid phase top at the time of the combination of this amino group and an epoxy group breathing out a nucleic-acid probe solution by the ink-jet ****** method as compared with the combination of a thiol group and a maleimide machine is not good, when the well is prepared in solid phase, it is the thing of the grade which can be disregarded.

[0062] The introduction to the solid phase front face of the amino group or an epoxy group As mentioned above, in using a glass plate as solid phase After processing this glass-plate front face with alkali, such as a potassium hydroxide and a sodium hydroxide, first and exposing a hydroxyl group (silanol group) on a front face, The amino group The silane coupling agent containing the silane compounds (for example, gamma-glycidoxypropyltrimetoxysilane etc.) which introduced the introduced silane compounds (for example, N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane etc.) and the epoxy group It can carry out by making it react with the hydroxyl group on this front face of a glass plate. Moreover, a maleimide machine can introduce N-maleimide KAPURO yloxy succinimide, SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate, etc. into a glass-plate front face by making it react with this amino group, after introducing the amino group into a glass-plate front face by the above-mentioned method.

[0063] In addition, the structure of N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane, gamma-glycidoxypropyltrimetoxysilane, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate is as follows.

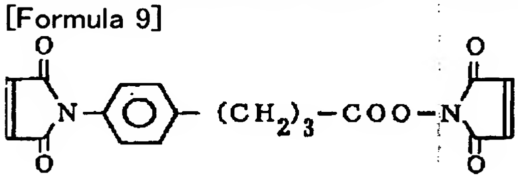
** N-beta (aminoethyl)-gamma-aminopropyl trimethoxysilane : (CH3O) it is 3H6NHC2H4NH2** gamma-glycidoxypropyltrimetoxysilane: [0064] 2 SiC.

[Formula 8]



[0065] ** SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate : [0066]

[Formula 9]



[0067] When an epoxy group is introduced into a solid phase front face in the surface treatment of the above-mentioned solid phase, after combining this epoxy group and a probe, the base of a well can be made into a hydrophilic property by carrying out ring breakage of the unreacted epoxy group using ethanolamine solution etc., and changing into a hydroxyl group. This operation is desirable when supplying this probe and the drainage system solvent containing the target matter which reacts specifically to the well which combined the probe.

[0068] Moreover, when using a resin substrate as solid phase, a hydroxyl group, a carboxyl group, or the amino group can be introduced into a resin substrate front face by the method of the publication of Chapter 5 of Organic Thin Films and Surface, Vol.20, and Academic Press. Or it is also possible to introduce the amino group and an epoxy group or to introduce a maleimide machine further using the silane compound which has an amino group and an epoxy group like the case of the glass plate described above after introducing a hydroxyl group by this method. By the way, introduction of the functional group to the above-mentioned solid phase may be performed before formation of a matrix on a solid phase front face, or you may perform it after matrix formation. What is necessary is just to supply a reaction solution to each well by the ink-jet method etc. that what is necessary is just to supply a reaction solution required for introduction of a functional group

to a solid phase front face on a solid phase front face by the method of a spin coat, a DIP coat, etc. if it is before matrix formation, if it is after matrix formation.

[0069] Moreover, oxidize a resin substrate front face, introduce a hydroxyl group, the silane coupling agent and this hydroxyl group containing the silane compound which subsequently has an amino group are made to react, the amino group is introduced, and the method to which this amino group and the functional group of a probe are made to react is mentioned as indicated by JP,60-015560,A as a method of combining a probe with a resin substrate.

[0070] Moreover, when the substrate after pretreatment is a hydrophilic property, the resin for the matrix formation made of a resin in which the matrix pattern of another side is formed and which spread a water-repellent material previously relatively can be used as it is. Moreover, when the further water repellence is needed, it can also add a water repellent in matrix material. Moreover, when a matrix pattern is formed by photopolymers, such as a photoresist, it is also possible by performing a postbake on suitable conditions after exposure and development to give strong water repellence with a matrix pattern.

[0071] Although the case where a probe solution was a hydrophilic property was described when saying so far which it was, what is necessary will be just to carry out reverse processing in a lipophilic case [a probe solution].

[0072] The size and the configuration of a well of a matrix pattern can be suitably chosen with the supply methods, such as size of a substrate, size of the whole array finally produced, the number of probe kinds that constitutes an array or the formation method of a matrix pattern, and a probe solution to a matrix pattern gap, the method of detection, etc.

[0073] A square configuration's can be alike, in addition the cross section of a field parallel to the substrate shown in drawing 5 as a configuration can consider as various configurations, such as a rectangle, various polygons, circular, and an ellipse form.

[0074] As size of a well, when the size of the number of reaction kinds and the whole array is taken into consideration, the longest width of face of 300 micrometers or less is desirable. For example, as shown in drawing 5 , when making the cross section in a direction parallel to the substrate of a well into a square, the length of one side can be set to 200 micrometers or less. Furthermore, in making a well into a rectangle, when making circular 200 micrometers or less of the long side, it is more desirable to set the diameter to 200 micrometers or less. The minimum of the size can be set to about 1 micrometer.

[0075] The array gestalt of each well can be suitably changed according to requests, such as a mode which is the vertical direction in a plan like drawing 5 , and is arranged at equal intervals, and a mode which shifts and arranges the position of a well in an adjacent train.

[0076] As for the distance between adjoining wells, it is desirable to set it as an interval which it does not make produce cross contamination even if a position gap of some arises between a regurgitation position and the well which should be supplied, in case a probe solution is supplied to a well for example, by the ink-jet method. Moreover, when the size etc. and cross contamination of the whole array, and the operability in the case of supply of various solutions are taken into consideration, it is desirable that the distance between adjoining wells is in the range of 1/2 of the longest width of face of a well - double precision.

[0077] For example, if the size of a substrate is made into a suitable size (1 inch x1 inch or 1cmx1cm) by the case where a well is made into a square configuration when automating operation of probe fixation, sample supply, detection, etc. From the need of fully achieving the function as combinatorial chemistry to 100 pieces x 100 pieces Or since it is desirable for the probe kind beyond 1000 piece x1000 piece to exist, it is desirable to set to 200 micrometers or less distance between the wells which adjoin one side of the square configuration of a well 1-200 micrometers also in consideration of the size of the matrix itself.

[0078] Moreover, although the thickness (height from a solid phase front face) of a matrix is determined in consideration of the capacity of the formation method of a matrix pattern, or a well, the amount of the probe solution to supply, etc., it is desirable to be preferably referred to as 1-20 micrometers. By considering as such thickness, namely, when [for example,] a probe solution is supplied to each well using an ink-jet regurgitation method, Even if it is the case where it can adjust only to a difficult property that this probe solution forms a small spot for the property of a probe solution on this solid phase front face, in relation with ink-jet regurgitation conditions The position on solid phase can be made to be able to stop this probe solution, and cross contamination can be prevented very effectively.

[0079] Temporarily, the capacity of the well in the upper limit of the above-mentioned desirable range becomes 200micrometerx200micrometerx20micrometer, i.e., 800pl. Moreover, if distance between adjoining

wells (x of drawing 1) is similarly set to 200 micrometers in this size, 625 well densities /of 2 will be obtained cm. That is, the array which has the well density of two or more [102 //cm] as order is obtained. Moreover, if a well is made into the square configuration whose one side is 5 micrometers, and distance between adjoining wells is also set to 5 micrometers and sets thickness of a matrix pattern to 4 micrometers, the capacity of a well will serve as 0.1pl(s) and 1 million [/] of the number will be set to 2 cm. since 5micrometerx5micrometerx4micrometer patterning is realistic size in the present ultra-fine processing technology — as order — the well of two or more [106 //cm] — the array of density may also serve as the range of invention of this invention

[0080] When a probe solution or a probe, and the supply volume to the well of the matter which should react consider as the amount of said mostly with the capacity of a well in this mode, it becomes a 0.1pico liter (pl) to a 1nano liter (nl) from the above-mentioned calculation in general. Moreover, when it considers as non-compatibility to the solution to which a matrix is supplied, depending on ****, it becomes possible to stop the liquid of the amount which exceeds the capacity of a well with the surface tension in the opening upper part of a well. In such a case, for example, 10 times of a well, volume 10 times the number of this can be supplied, and it can be made to hold. That is, the liquid of number 10nl will be supplied from Number pl. It is desirable in any case, to supply a probe solution at a well using the ink-jet method which can supply position precision and amount-of-supply precision good, although a general micro dispenser and a general micropipette are also possible for the supply to such a little well of liquid. On an ink-jet print, since it positions with high precision to mum order and the regurgitation of the ink is carried out, it can be told to supply of the solution to a well that it is extremely suitable. moreover, the amount of the ink breathed out — general — several — from pl, since it is number 10nl, it can be said that it is suitable for supply of the solution to a well also at this point

[0081] If a drop adheres to the field containing a well even if according to this mode the breadth of a drop is quantitatively controlled by the reaction and well on a nucleic-acid probe and the front face of solid phase and disorder of some is in a discharge direction, the portion will be crawled and the portion concerning the matrix of a drop will be smoothly contained in a well, when the matrix serves as non-compatibility to regurgitation liquid.

[0082] Although especially the ink-jet method used for this invention is not restricted, a piezo jet process, the bubble jet process using thermal foaming, etc. can be used, for example.

[0083] By the way, what can form a matrix in a front face in the 2nd mode further is [that what is necessary is just what can introduce various functional groups which were described above on the solid phase front face as a material which can be used as solid phase 103 in this invention] desirable. And when constructing the detection system which minded solid phase when the reactant of a probe and the target matter was detected optically, it is desirable to make solid phase into transparent solid phase optically. The glass which contains synthetic quartz, a fused quartz, etc. as such a material, silicon, acrylic resin, polycarbonate resin, polystyrene resin, vinyl chloride resin, etc. are mentioned. Moreover, when detecting optical detection of this reactant without minding solid phase, it is desirable to use black solid phase optically, and the resin substrate containing black stain pigments, such as carbon black, etc. is used.

[0084] In this invention, the solution of the matter which should react to these probes array is supplied, and it reacts by putting on a suitable reaction condition. When the solution of matter which is different in an individual well and which should react needs to be supplied, at least one sort of solutions which at least one sort of matter which should be made to react to a probe dissolved in each of two or more wells of a probe array are supplied. In this case, supply of the quantitive liquid without cross contamination which limited the supply field when it was affinity-like to the well with which the probe of a probe array with which the solution supplied is already formed is combined and was un-keeping-good relations-like [a matrix pattern] is attained as mentioned above. Since many of living body-related matter is water-soluble like the matter shown in Table 1, in a well, in this case, a hydrophilic property and a matrix pattern serve as water repellence. Moreover, as mentioned above, if the ink-jet method is used also for supply of these matter that should react, supply will become possible quantitatively about minute amount volume at it.

[0085] Since the volume of the probe supplied in this invention in order to combine with a substrate, or the volume of the matter which should react is a minute amount, it is desirable to include the conditions from which both reaction conditions protect evaporation of the supplied solution and ****.

[0086] this invention is explained still in detail with an example below.

[0087] Example 1 (the process of the nucleic-acid probe array using the bubble-jet printer, and evaluation of the probe array)

(1) The glass plate of the 1 inch angle of substrate washing was put into the rack, and it dipped in the detergent for ultrasonic cleaning overnight. Then, ultrasonic cleaning was performed for 20 minutes in the detergent, and rinsing removed the detergent after that. It was distilled water, and after rinsing, it ultrasonicated for 20 minutes further in the container containing distilled water. Next, the glass plate was dipped in 1-N sodium-hydroxide solution beforehand warmed at 80 degrees C for 10 minutes. Rinsing and distilled water washing were performed succeedingly and the glass plate for probe arrays was prepared.
[0088] (2) The 1wt% solution of the silane coupling agent (tradename : KBM603; Shin-Etsu Chemical [Co., Ltd.] Co., Ltd. make) containing the silane compound (N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane) which combined the surface treatment amino group was stirred under the room temperature for 2 hours, and the methoxy machine in the molecule of the above-mentioned silane compound was hydrolyzed. Subsequently, after dipping the substrate obtained above (1) in this solution for 20 minutes at a room temperature (25 degrees C), it pulled up and both sides of a glass plate were made to spray and dry nitrogen gas. Next, for 1 hour, silane coupling processing was completed, and the amino group was introduced into the substrate front face. [in the oven which heated the glass plate at 120 degrees C] Subsequently, 2.7mg weighing capacity of the N-maleimide KAPURO yloxy succinimide (product made from N-(6-Maleimidocaproyloxy) succinimide;Dojin) (it abbreviates to EMCS henceforth) was carried out, and the EMCS solution which dissolved so that the last concentration might become 1:1 solutions of dimethyl sulfoxide (DMSO)/ethanol in ml and 0.3mg /was prepared. The glass plate which performed silane coupling processing was dipped in this EMCS solution at the room temperature for 2 hours, and the amino group currently supported by silane coupling processing on the glass-plate front face and the carboxyl group of an EMCS solution were made to react. The maleimide machine of the EMCS origin will exist in a glass-plate front face on a front face in this state. After washing the glass plate pulled up from the EMCS solution one by one by the mixed solvent of DMSO and ethanol, and ethanol, it was dried under nitrogen-gas-atmosphere mind.
[0089] (3) The single strand nucleic acid of the array number 1 was compounded using the synthetic DNA automatic composition machine of probe DNA. In addition, the thiol (SH) machine was introduced by using a thiol modifier (Thiol-Modifier) (grain research (GlenResearch) company make) for the single stranded DNA end of the array number 1 at the time of composition with a DNA automatic composition machine. Then, the usual deprotection was performed, DNA was collected, and it refined in the high performance chromatography, and used for the following experiments.
Array number: The single stranded DNA of the DNA regurgitation by the 15 'HS-(CH2)6-O-PO2-O-ACTGGCCGTCGTTTTACA3' (4) BJ printer and the joint above-mentioned array number 1 to a substrate was dissolved in TE solution (10mM Tris-HCl (pH 8) / 1mM EDTA solution) so that the last concentration might become [ml] in about 400mg /, and the single stranded DNA solution was prepared (exact concentration is computed from absorption intensity).
[0090] Glycerol 7.5wt%, urea 7.5wt%, the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% shown by the thiodiglycol 7.5wt% and above-mentioned general formula (I) was prepared, and in addition to the above-mentioned DNA solution, it adjusted so that the last concentration of a single stranded DNA might be set to 8microM. The surface tension of this liquid was within the limits of 30 - 50 dyne/cm, and viscosity was 1.8cps (E type viscometer : Tokyo Keiki [Co., Ltd.] Co., Ltd. make). The ink tank for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) was filled up with this liquid, and the bubble jet head was equipped with it. In addition, the bubble-jet printer (tradename : BJC620; Canon [, Inc.], Inc. make) used here converts so that printing to a plate may be possible. Moreover, this bubble-jet printer is printable in the resolution of 360x720dpi. Subsequently, this printer was equipped with the glass plate processed above (2), and the liquid containing a probe nucleic acid was spotted on the glass plate. The distance of the liquid regurgitation side of a bubble jet head and the liquid adhesion side of a glass plate was 1.2-1.5mm here. Moreover, spotting performed 2 times of empty regurgitation in the direction of 360dpi after one spotting, and conditioning was carried out in the direction of 720dpi so that 5 times of empty regurgitation might be performed after one spotting. The glass plate was put into the humidification chamber for 30 minutes after the spotting end, and the maleimide machine on the front face of a glass plate and the thiol group of a nucleic-acid probe end were made to react. In addition, the discharge quantity of the DNA probe solution per 1 discharging of the above-mentioned printer was about 24 pl(s).
[0091] (5) It is 1M after a reaction end with a blocking reaction maleimide machine and a thiol group, and about a glass plate. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the liquid containing

DNA on the front face of a glass plate was flushed completely. Subsequently, the glass plate was dipped into 2% bovine-serum-albumin solution, it was left for 2 hours, and the blocking reaction was performed.

[0092] (6) DNA of the hybridization reaction array number 1 and the single stranded DNA which has a complementary base sequence were compounded with the DNA automatic composition machine, and the single stranded DNA which was made to combine a rhodamine with a five prime end, and labeled was obtained. It is 1M about this labeling single stranded DNA. It dissolved so that it might become the last concentration M of 1micro at NaCl / 50mM phosphate buffer solution (pH 7.0), and the probe array which was obtained above (5) in this solution and which carried out blocking processing was immersed, and the hybridization reaction was performed at the room temperature (25 degrees C) for 3 hours. Then, it is 1M about a probe array. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the probe nucleic acid and the single stranded DNA which was not hybridized were flushed. Next, the fixed quantity was carried out using the done type fluorescence microscope equipped with the filter set which connects image-analysis equipment (tradename : ARGUS 50; Hamamatsu Photonics make), and fits Rhodamine B in the amount of fluorescence of the spot of this probe array of a handstand.

[0093] (7) At the spot of the nucleic-acid probe of the array number 1 which are a resulting-indicator-ized single stranded DNA and a full match, it was the amount of fluorescence of 4600. Moreover, the probe array in the state where each spot after hybridization is carrying out firefly luminescence was observed using the fluorescence microscope (NIKON [CORP.] CORP. make). the probe array which starts this example as a result — a — between that it is in within the limits the diameter of whose each spot is almost circular and is about 70-100 micrometers, and the spots of which b contiguity is done — the diameter of each spot, and abbreviation — there is about 100-micrometer equal space and it became clear that each spot has been independent clearly mutually and that the row and column of c spot has gathered

[0094] This is very effective when making automatic detection of the spot hybridized on the probe array etc. perform.

[0095] Example 2 (manufacture of the nucleic-acid probe array using the bubble-jet printer, and detection of the target-nucleus acid using the probe array)

(1) The glass plate which performed surface treatment for probe arrays completely like (1) of the above-mentioned example 1 and (2) was prepared.

[0096] (2) The single strand nucleic acid of the array numbers 1-4 was compounded using the synthetic DNA automatic composition machine of probe DNA. In addition, the array number 2 and the thing which carried out 3 base change were made into the array number 3, and what was changed six bases was made into the array number 4 for what carried out 1 base change on the basis of the array number 1 which used the single strand nucleic acid of the array numbers 1-4 in the example 1. Moreover, the thiol (SH) machine was introduced by using Thiol-Modifier (product made from GlenResearch) for the single stranded DNA end of the array numbers 1-4 at the time of composition with a DNA automatic composition machine. Then, the usual deprotection was performed, DNA was collected, and it refined in the high performance chromatography, and used for the following experiments. The array of the array numbers 2-4 is shown below.

Array number : 25'HS- 6-O-PO2-O-ACTGGCCGTTGTTTTACA3' array number : 35'HS-(CH2)6-O-PO2-O-ACTGGCCGCTTTTTTACA3 (CH2) 'array number : The regurgitation of the DNA probe by the 45 'HS-(CH2) 6-O-PO2-O-ACTGGCATCTTGTTTACA3' (3) BJ printer, And four kinds of liquids for regurgitation are prepared using the single stranded DNA of the joint above-mentioned array numbers 1-4 to a substrate by the method indicated to (4) of the above-mentioned example 1, and the same method. Four ink tanks for bubble-jet printers used in the example 1 were filled up with each liquid, and the bubble jet head was equipped with each ink tank. Subsequently, this printer was equipped with the glass plate created by the same method as the above (1), and each of four sorts of nucleic-acid probes was spotted on this glass plate at 3x3mm each of four area of this glass plate. In addition, the pattern of spotting in each area presupposed that it is the same as that of an example 1. The glass plate was put into the humidification chamber for 30 minutes after the spotting end, and the maleimide machine and the thiol group were made to react.

[0097] (4) It is 1M after a reaction end with a blocking reaction maleimide machine and a thiol group, and about a glass plate. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the DNA solution on the front face of a glass plate was flushed completely. Subsequently, the glass plate was dipped into 2% bovine-serum-albumin solution, it was left for 2 hours, and the blocking reaction was performed.

[0098] (5) DNA of the hybridization reaction array number 1 and the single stranded DNA which has a complementary base sequence were compounded with the DNA automatic composition machine, the

rhodamine was combined with the five prime end, and the labeling single stranded DNA was obtained. It is 1M about this labeling single stranded DNA. It dissolved so that it might become the last concentration M of 1micro at NaCl / 50mM phosphate buffer solution (pH 7.0), and the probe array and hybridization reaction which were obtained by (4) were performed for 3 hours. Then, it is 1M about a probe array. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the probe nucleic acid and the single stranded DNA which was not hybridized were flushed. Next, each spot of this probe array was observed with the fluorescence microscope (NIKON [CORP.] CORP. make), and the fixed quantity was carried out using the done type fluorescence microscope equipped with the filter set which connects image-analysis equipment (tradename : ARGUS 50; Hamamatsu Photonics make), and fits Rhodamine B in the amount of fluorescence of a handstand.
[0099] (6) The amount of fluorescence of 2800 was obtained at the spot of the DNA probe of the array number 2 which has the mismatch array of one base to being the amount of fluorescence of 4600 at the spot of the DNA probe of the array number 1 which are a resulting-indicator-ized single stranded DNA and a full match. Moreover, at the spot of the DNA probe of the array number 3 which has 3 base mismatch, only the amount of fluorescence below the half of 2100 and a full match was obtained, and fluorescence was not observed in DNA of the array number 4 of 6 base mismatch. From the above thing, the single stranded DNA of a perfect complementarity was specifically detectable on the DNA array substrate.
[0100] Example 3 (the concentration and the bubble jet regurgitation property of the DNA probe in a liquid)
(1) The single stranded DNA which has the array of the array number 5 shown below in composition of a DNA probe was compounded using the DNA automatic composition machine, it was dissolved in TE solution (10mM Tris-HCl (pH 8) / 1mM EDTA solution) so that concentration might become [ml] respectively in about 0.2mg [ml] /, 2mg [ml] /, and 15mg /, and three kinds of DNA probe solutions with which concentration differs were prepared (exact concentration was computed from absorption intensity).
Array number : Regurgitation glycerol 7.5% by the 55'GCCTGATCAGGC3'(2) BJ printer, The solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1% which has the structure shown by the above-mentioned general formula (I) is prepared 7.5% of ureas, and thiodiglycol 7.5%. In addition to the probe solution with a concentration of 0.2mg [/ml] adjusted above (1), the last concentration diluted [ml] this solution in about 0.02mg (3microM) /. The ink tank for bubble-jet printers which used this liquid in the above-mentioned example 1 was filled up, and the head of the bubble-jet printer which used this ink tank in the example 1 was equipped.
[0101] Next, this printer was equipped with the aluminum board of A4 size, and it spotted to the area of the 3x5 square inches of this aluminum board. Spotting here was set up so that the above-mentioned area might spot by the density of 360x720dpi. Moreover, the ink of marketing [to the beginning] for BJ620 as control was printed on this aluminum board. This operation was performed to a total of four aluminum boards.
[0102] Next, the nucleic-acid probes by which the spot was carried out on each aluminum board were collected using TE solution, it refined by gel filtration technique and the amount of the refined recovery nucleic-acid probe was measured with the absorption spectrum. The amount of recoveries of the nucleic-acid probe called for theoretically here is as follows. That is, the volume per [which is breathed out from the head of the printer used for this example] drop is a 24pico liter. And since there are four aluminum boards which spotted in the area of 3x5 square inches by the density of 360x720dpi, it is set to 24(pico liter) x(720x360) x (3x5) x four-sheet =373microl. The absorbance in 260nm which the probe nucleic acid of this amount shows, and the absorbance in 260nm of the collected nucleic-acid probe are shown in drawing 3 .
[0103] In the completely same operation as the above (2), 15mg /followed each probe solution ml the concentration of 2mg/ml. In addition, the last concentration of the nucleic-acid probe of each liquid for regurgitation was set to 30microM (0.2mg/(ml)) and 225microM (1.5mg/(ml)). The result of the absorbance which the absorbance and the amount of probe nucleic acids calculated theoretically which the probe nucleic acid collected from each solution shows show is shown in drawing 3 .
[0104] (3) It was a value near the value with which the discharge quantity with an actual nucleic-acid probe is theoretically expected that result drawing 3 shows. In the regurgitation of the nucleic-acid probe using a bubble jet process, quantitative loss of the nucleic-acid probe by the bad debt of the nucleic-acid probe to the heater section of a bubble jet head etc. is not accepted from this thing. Moreover, the spotting in process to the aluminum board using the liquid of each concentration and no trouble of a head, for example, the non-regurgitation etc., were generated. Moreover, when the spot of the ink for bubble-jet printers and the spot of a nucleic-acid probe which spotted to the aluminum board as control were contrasted visually, the spotting

situation of the spot created using the liquid of concentration 3microM and 30microM was almost the same as that of it of an ink spot. Moreover, as for the spot created using the liquid with a concentration [M] of 225micro, some disorder was accepted as compared with the ink spot.
[0105] Example 4 (examination of the influence which a bubble jet process has on a nucleic-acid probe)
(1) It prepared so that base length 10mer (synthetic compounds), oligoA (40-60mer; Pharmacia manufacture) and poly (dA) which consist of a synthetic adenine (it is henceforth indicated as "A") of a nucleic-acid probe, and a (300-400mer; Pharmacia manufacture) might be diluted with TE solution, respectively and the last concentration might become [ml] in 1mg /, and the nucleic-acid probe solution with which length differs was prepared. In addition, the base sequence (array number : 6) of 10mer(s) is as follows.
Array number: Regurgitation glycerol 7.5wt% of the DNA solution by the 65" [AAAAAAAAA3] (2) bubble-jet printer, the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals) 1wt% shown by the urea 7.5wt% and above-mentioned general formula (I) was prepared, and each nucleic-acid probe solution created above (1) in this solution was diluted so that the last concentration might become in ml and about 0.1mg /.
[0106] Each nucleic-acid probe solution with which the cartridge was filled up like the example 3 was made to breathe out on an aluminum board, and the spotting situation was observed visually. As a result about the nucleic-acid probe of base length 10mer and 40-60mer, the probe array with which the spot which became independent on the aluminum board was tidily located in a line was obtained. Moreover, although the same probe array was fundamentally obtained also about the nucleic-acid probe of 300-400mer, the portion with which adjoining spots are connected was accepted. Physical-properties change of the liquid with which the base chain of a nucleic-acid probe originates in a ******** arises, and this is considered because the directivity of the regurgitation from a bubble jet head became incorrectness a little.
[0107] Next, the spots on the probe array created using each nucleic-acid probe solution were collected like the example 3. 100micro of collected nucleic-acid probe solutions l was analyzed by Antiphase HPLC, and comparison with the solution in front of the regurgitation investigated the existence of cutting of a nucleic-acid probe. In addition, 7 - 70% acetonitrile concentration gradient containing 1M triethylamine acetate performed elution of Antiphase HPLC. Consequently, a DNA fragment which is considered to have been cut was not observed but, therefore, it has checked that the nucleic-acid probe had not received transformation by the regurgitation in a bubble jet process, either. Moreover, as a result of performing the fixed quantity of the collected nucleic-acid probe like an example 3, as shown in drawing 4 , as for the nucleic-acid probe of three kinds of length, the amounts as a theoretical value were collected mostly.
[0108] In (4) of example 5 (examination of reaction time) example 1, except having carried out room temperature (25 degrees C) neglect of the surface treatment glass plate which spotted the nucleic-acid probe into the humidification chamber overnight for 10 minutes and 90 minutes, the probe array was manufactured like the example 1 and the hybridization reaction was presented with each probe array. About the probe array made to react as a result 90 minutes and overnight, fluorescence intensity of the same grade as the fluorescence intensity which the probe array altogether obtained in the example 1 shows was given. It became clear to have ended mostly the ligation reaction of the maleimide machine on the front face of a glass plate and the thiol group of a nucleic-acid probe end in 30 minutes from this. On the other hand, the reaction time of the probe array for 10 minutes was about 70% of the amount of fluorescence compared with it of an example 1.
[0109] Example 6 (manufacture of the PNA probe array using the bubble-jet printer, and detection of the target-nucleus acid using the probe array)
(1) The glass plate which performed surface treatment for probe arrays completely like (1) of the above-mentioned example 1 and (2) was prepared.
[0110] (2) The protein nucleic acid (PNA) (product made from Japanese par SEPUTIBU) which has the base sequence of the synthetic following array numbers 7 and 8 of Probe PNA was prepared. A cysteine residue (Cys and notation) is combined with an amino terminus (equivalent to the five prime end of DNA), and, as for this PNA, the thiol group is introduced into the amino terminus as the result. Moreover, the PNA probe of the array number 8 little salt-machine-changes the PNA probe of the array number 7.
Array number : 7 NCys-NH 2-O- (CH2) 2-O-CH2 CONH-ACTGGCCGTCGTTTTACAC array number : The regurgitation of the PNA probe according to a 2-O-(CH2)2-O-CH2 CONH-ACTGGCCGTTGTTTTACAC(3) BJ printer 8 NCys-NH (CH2), (CH2) And the PNA probe of each joint above to a substrate is dissolved so that the last concentration may be set to 80microM at the 0.1wt% trifluoroacetic acid of 100microl.

Subsequently, glycerol 7.5wt% and urea 7.5wt% and thiodiglycol 7.5wt%, and the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% shown by the above-mentioned general formula (I) — the trifluoroacetic-acid solution of Above PNA — in addition, it adjusted so that the last concentration of a PNA probe might be set to 8microM The surface tension of this liquid was within the limits of 30 - 50 dyn/cm, and viscosity was within the limits of 1-5cps.

[0111] It spotted in each area on the glass plate which created these PNA probe solutions of each above (1) the same with having indicated to (3) of an example 2. It put after a spotting end and into the 3-hour humidification chamber, and the maleimide machine and the thiol group were made to react.

[0112] In addition, the discharge quantity of the PNA probe solution per 1 discharging of the above-mentioned printer was about 24 pl(s).

[0113] (4) It is 1M after a reaction end with a blocking reaction maleimide machine and a thiol group, and about a glass plate. NaCl / 50mM phosphate buffer solution (pH 7.0) solution washed, and the liquid containing PNA on the front face of a glass plate was flushed completely. Subsequently, the glass plate was dipped into 2% bovine-serum-albumin solution, it was left for 3 hours, and the blocking reaction was performed.

[0114] (5) The single stranded DNA which has PNA of the hybridization reaction array number 7 and a complementary base sequence was compounded with the DNA automatic composition machine, and the single stranded DNA which was made to combine a rhodamine with a five prime end, and labeled was obtained. This labeling single stranded DNA was dissolved so that it might become 10mM phosphate buffer solution (pH 7.0) with last concentration 5nM (the amount of solutions of 1ml), the PNA probe array which was obtained above (4) in this DNA solution and which carried out blocking processing was immersed, and the hybridization reaction was performed at the room temperature (25 degrees C) for 12 hours. Then, 10mM phosphate buffer solution (pH 7.0) solution washed the probe array, and the PNA probe and the single stranded DNA which was not hybridized were flushed. Next, the fixed quantity was carried out using the done type fluorescence microscope equipped with the filter set which connects image-analysis equipment (tradename : ARGUS 50; Hamamatsu Photonics make), and fits Rhodamine B in the amount of fluorescence of the spot of this probe array of a handstand.

[0115] (6) In the PNA probe of the array number 8 which has 1 base mismatch array to having been the amount of fluorescence of 2400 in the PNA probe of the array number 7 which are a resulting-indicator-ized single stranded DNA and a full match, it was 1100 of an abbreviation half. The single stranded DNA of a perfect complementarity was specifically detectable on the PNA array from the above thing.

[0116] Moreover, the probe array in the state where each spot after hybridization is carrying out firefly luminescence was observed using the fluorescence microscope (NIKON [CORP.] CORP. make). the probe array which starts this example as a result — a — about 50-micrometer space is between that it is in within the limits the diameter of whose each spot is almost circular and is about 200 micrometers, and the spot of which b contiguity is done, and it became clear that each spot has been independent clearly mutually and that the row and column of c spot has gathered

[0117] This is very effective when making automatic detection of the spot hybridized on the probe array etc. perform.

[0118] Furthermore, since there was no need of making hybridization reaction time and the solution used for removal of a subsequent unreacted single stranded DNA containing a sodium chloride, it did not need to be cautious of a deposit of a sodium chloride during observation of fluorescence, and the hybrid on a probe array was able to be detected more easily. Moreover, a preservation top did not have the need for seal, either and was easy handling.

[0119] In addition, although a larger reason than the spot of the probe array which the diameter of a spot of a PNA probe obtained in the example 1 is not clear, as a result of having acquired knowledge that water solubility is inferior in a PNA probe a little as compared with a DNA probe and both water-soluble difference making the surface tension of each ink-jet regurgitation liquid produce a difference, as for this invention persons, it is guessed that the diameter of a spot is a different thing.

[0120] Example 7 (manufacture and its evaluation of the glass substrate with a black matrix for probe arrays which introduced the epoxy group into the front face)

(1) The glass substrate (50mmx50mm) which consists of synthetic quartz was cleaned ultrasonically using 2wt% sodium-hydroxide solution, subsequently UV ozonization was performed, and the front face was defecated. the silane coupling agent (tradename : KBM403; Shin-Etsu Chemical Co., Ltd. make) containing the silane compound (gamma-glycidoxypropyltrimetoxysilane) which combined the epoxy group — 1wt% — the

50wt% methanol solution to contain was stirred under the room temperature for 3 hours, and the methoxy machine in the above-mentioned silane compound was hydrolyzed Subsequently, this solution was applied to the above-mentioned substrate front face by the spin coater, it heated and dried for 5 minutes at 100 degrees C, and the epoxy group was introduced into the substrate front face.
(2) The DEEP-UV resist (negative resist for black matrices) (tradename : BK- 739P; the NIPPON STEEL chemistry incorporated company make) which next contains carbon black is applied so that the thickness after hardening may be set to 5 micrometers by the spin coater, and with the hot plate, at 80 degrees C, this substrate was heated for 5 minutes and stiffened. Pro squeak tee exposure was carried out using the mask by which patterning was carried out so that the distance between the contiguity wells in drawing 5 (X) might serve as 100 micrometers and a square whose configuration of a well is 100micrometerx100micrometer to a 1cmx1cm field using a DEEP-UV aligner, subsequently, negatives were developed with the developer of an inorganic alkaline-water solution using the spin developing machine, pure water washed further, and the developer was removed completely. Next, it dried simply using the spin dryer, it heats for 30 minutes at 180 degrees C in clean oven after that, and actual hardening of the resist was carried out, 2500 wells have been arranged in the predetermined array and the adjoining well obtained the substrate isolated by the black matrix. In addition, the capacity of each well is calculated with a 50pico liter (pl). Due to this time, the contact angle to the water of a black matrix front face cannot get wet easily with 93 degrees, and the contact angle to the water at the base of a well tended to get wet with 35 degrees.
[0121] (3) The ink tank for bubble-jet printers (tradename : BJC620 : Canon [, Inc.], Inc. make) was filled up with the Rhodamine B solution of 10microM, and the bubble jet head of the bubble-jet printer used in the aforementioned example 1 was equipped. and the printer was equipped with the solid phase prepared by the above (1) and (2), and Rhodamine B solution was supplied to the check pattern (even — setting) at the well of this solid phase In addition, the amount of supply per one well is about 50 pl(s). Moreover, the regurgitation positioning accuracy of this printer is **2.5 micrometers. Next, another ink tank was filled up with the solution of 10microM amino [FITC], the bubble jet head of the above-mentioned printer was equipped, and another well which adjoins the well which supplied Rhodamine B solution previously was supplied. It is water-soluble to have used Rhodamine B and amino one FITC here, and it is because the regurgitation from an ink-jet head being performed easily, and the state and cross contamination of a liquid which were supplied to the well by observation of fluorescence can be checked.
[0122] (4) The fluorescence microscope (NIKON [CORP.] CORP. make) was equipped with G excitation filter (for Rhodamine B), and B excitation filter (for amino FITC), and the state of each solution supplied to the well by one 100 times the scale factor of this was observed by fluorescence. As a result, each solution was uniformly supplied in the well, without forming a drop. Moreover, mutually from each well, the fluorescence of other coloring matter was not observed and cross contamination was not accepted.
[0123] Example 8 (manufacture of the probe array using the substrate of an example 7, and detection of the target-nucleus acid using it)
(1) The substrate with a black matrix (BM) was created by the same method as an example 7.
(2) two nucleotides prepared [one nucleotide] the probe (array number : 11) (all — the Nippon Flour Mills Co., Ltd. make and HPLC grade) of a mismatch to the probe (array number : 10) of a mismatch, and the oligomer of the array number 9 to the oligomer (array number : 9) of 18 **** which combined the amino group with the hydroxyl group of a five prime end through the phosphoric-acid machine and the hexamethylene as a DNA probe, and the oligomer of the array number 9 The base sequence of the oligomer of the array number 9 is an array complementary to some base sequences of the multiple cloning site of M13mp18-ssDNA which is a single stranded DNA. the following — array number: — the base sequence of 9-11 and the structure of linkage are shown
array number: — 95 — 'NH2-(CH2)6-O-PO2-O-TGTAAAACGACGGCCAGT3' — array number: — 105 — 'NH2-(CH2)6-O-PO2-O-TGTAAAACCACGGCCAGT3' — array number: — 115 — 'NH2-(CH2)6-O-PO2-O-TGTATAACCACGCCCAGT3' — the DNA probe of the (3) above-mentioned array numbers 9-11 — receiving — completeness — the complementary single stranded DNA was compounded Next, each DNA probe and the single stranded DNA were dissolved in TE solution (pH 8) which contains NaCl by the concentration of 50mM (s) so that the last concentration might be set to 100microM, and the DNA probe solution and the single stranded DNA solution were prepared. And the solution which contains a complementary single stranded DNA in each DNA probe to 100micro of solutions l containing a DNA probe was 100microl Added, it mixed, each mixed solution was linearly cooled over 2 hours from 90 degrees C to 25 degrees C, and the hybrid of each

DNA probe and each single strand nucleic acid was made to form. Next, the above-mentioned array number: In addition to the solution containing acetylene alcoholic (tradename : ASECHIRE Norian EH; Kawaken Fine Chemicals [Co., Ltd.] Co., Ltd. make) 1wt% shown by the thiodiglycol 7.5wt% and aforementioned general formula (I) glycerol 7.5wt% and urea 7.5wt% in the solution containing the hybrid of each DNA probe of 9-11, it adjusted so that the hybrid last concentration might be set to 8microM. Each surface tension of these liquids containing the hybrid of each DNA probe was within the limits of 30 - 50 dyne/cm, and viscosity was also within the limits of 1-5cps (E type viscometer : Tokyo Keiki [Co., Ltd.] Co., Ltd. make).

[0124] Next, three ink tanks for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) were prepared, each ink tank was filled up with three sorts of above-mentioned hybrid solutions, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the glass substrate with BM created by the above (1) and (2) was set, and the solution which contains the hybrid of the DNA probe of the array number 9 first was supplied to the well (131 of drawing 6) of eye one train. Next, the solution containing the hybrid of the DNA probe of the array number 10 was supplied to 2 **** well (133 of drawing 6) which adjoins the well of eye the one above-mentioned train, and the solution which contains the hybrid of the DNA probe of the array number 11 further was supplied to the well (135 of drawing 6) of eye three trains adjoin the well of eye the two above-mentioned train. In addition, to one well, the regurgitation of which hybrid solution was carried out 4 times, and about 100 pl supply was carried out. Although the supplied hybrid solution rose from opening of a well and it existed when each well was observed under the microscope, although this amount was twice [about] the capacity of one well, it has stopped in the well by the hydrophobic matrix, and the cross contamination between wells was not observed.

[0125] next, a substrate — the constant temperature of 25 degrees C and 100% of humidity — the amino group of every 12 hours and a probe and the epoxy group of a well were made to react to a constant humidity chamber In addition, since the amino group of the base of a probe forms a complementary perfect single stranded DNA and a perfect complementary hybrid, it does not react with the epoxy group of each well.

[0126] (4) Next, 80-degree C pure water washed the substrate for 10 minutes, and while making the complementary strand which has constructed the probe combined with the substrate, and the hybrid dissociate from a probe, it washed away. Subsequently, the substrate was processed under the room temperature in ethanolamine solution 1% for 1 hour, and ring breakage of the unreacted epoxy group in each well was carried out. Next, pure water washed the substrate and it dried.

[0127] Since ring breakage of the DNA probe in a well and the epoxy group which did not react is carried out, and it turns into a hydroxyl group by **** of the above (4) and a hydroxyl group exists also in the ethanolamine made to react, a hydrophilic property becomes high more and the base of a well becomes advantageous in the case of supply to the well of the solution containing the below-mentioned target single stranded DNA.

[0128] (5) The single stranded DNA of a perfect complementarity to the DNA probe of the array number 9 was dissolved in TE solution (pH 8) which next contains NaCl by the concentration of 50mM(s) so that the last concentration might be set to 10microM, it was immersed, the probe array which introduced the epoxy group into the well obtained above (4) in this solution was lowered over 2 hours from 80 degrees C to 25 degrees C, and the high buri tie ZESHON reaction was performed. Subsequently, after TE buffer solution (pH 8) which contains NaCl of 10mM(s) at 20 degrees C washed the substrate for 20 minutes, the spin **** machine removed the surface penetrant remover.

[0129] (6) The 2-methyl -4 and 6-screw (4-N and N-dimethylamino phenyl) pyrylium eye OTAIDO (it abbreviates to "P2" below) which emit fluorescence only after intercalate in a double strand nucleic acid were dissolved in TE solution (pH 8.0) which next contains NaCl by the concentration of 50mM(s) so that the concentration might be set to 10microM, the ink tank for the above-mentioned ink jet printers was filled up with this solution, and it was attached in the head of the above-mentioned ink jet printer. Moreover, the substrate which performed hybridization above (5) is set to the above-mentioned printer. After supplying 100 pls of P2 solutions at a time to each well, in order to prevent dryness, it is left for 5 minutes within the exclusive chamber of 100% of humidity. having held in a chamber — a done-a handstand type microscope (tradename: — IMT2; Olympus optical incorporated company make —) Scale factor: 100 times, the filter cube for fluorescence microscopes (595nm (transparency) from filter 455nm for excitation) An ICCD camera (tradename : C2400- 87; Hamamatsu Photonics make) and an image processor (tradename : ARGUS 50; Hamamatsu Photonics make) are connected to use for dichroic mirror 620nm and barrier filter 610nm for fluorescence to 725nm (transparency). The observation fixed quantity of the fluorescence was carried out. In

addition, observation area is 25micrometerx25micrometer, integration x64, and ARGUS. The amplification level of 50 was set up suitably.
[0130] Consequently, from the well which combined the DNA probe of the array number 11, the almost same fluorescence intensity of 1200-1500 as the background was observed. The fluorescence intensity of 3500-3900 was observed from the well which the fluorescence intensity of 9800-10300 is observed [well] from the well which, on the other hand, combined the DNA probe of the array number 9, and combined the DNA probe of the array number 10. Furthermore, when each solid phase is washed for 10 minutes at 35 degrees C using TE buffer solution and fluorescence intensity is measured again, from the well which combined the DNA probe of the array number 10, only fluorescence intensity of the same grade as the background is observed.
[0131] These results showed that a hybridization reaction could be performed in each well and the array number 9 and a perfect complementary target-nucleus acid could be further detected specifically by using the probe array concerning this example.
[0132] Example 9 (alternative supply of the reacting matter to each well of the probe array of an example 8, and reaction with a probe)
(1) The substrate which combined the DNA probe of the array numbers 9-11 like the example 8 was prepared.

[0133] (2) Three kinds of perfect complementary single stranded DNAs were compounded to the DNA probe of the array numbers 9-11. The three above-mentioned kinds of single stranded DNAs were dissolved in TE solution (pH 8) which contains NaCl by the concentration of 50mM(s) so that each concentration might be set to 100microM. Three ink tanks for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) were prepared, each ink tank was filled up with three sorts of above-mentioned single stranded DNA solutions, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the substrate prepared above (1) was also set to the printer, and supplied every 100 pls per well of solutions which contain a perfect complementary single stranded DNA respectively to the well which the DNA probe of the array numbers 9-11 has combined. When the state of each well was observed under the microscope at this time, it turns out that the solution of the matter which bleeding of liquid and cross contamination are not observed and should be made to react to each well of a probe array individually can be supplied.
[0134] (3) After making a hybridization reaction perform in each well like an example 8 next, P2 solution was supplied to each well like the example 8, and the hybrid was detected by observing fluorescence. Consequently, the fluorescence of the intensity of 9800-10300 was observed from all wells. Supplied the reacting matter to each well of a solid phase probe array individually from this, the probe and the reacting matter were made to react in each well, and it was checked that an object is detectable as a result of a reaction.
[0135] Example 10 (hydrophilicity-ized processing at the base of a well of the substrate of an example 7)
(1) The glass substrate which has a black matrix pattern like an example 7 was prepared.
[0136] (2) UV ozonization was performed on the near front face in which the black matrix of this substrate is formed. At this time, the contact angle to the water of a black matrix front face was in 93 degrees and the state of being hard to get wet, and the contact angle to the water at the base of a well was 22 degrees, and was in the state of being easy to get wet as compared with it at the base of a well of the substrate with a black matrix obtained in the example 7. This is considered to be an effect by the above-mentioned UV ozonization.
[0137] (3) When the supply situation of the ink-jet regurgitation liquid to a well was observed using Rhodamine B and solution amino [FITC] like the example 7 next, each solution of both was uniformly supplied in the well, without forming a drop within a well. Unlike the case where the solid phase which does not have a well on a front face in using the solid phase which equipped the front face with the well as solid phase of a probe array and which has a flat and uniform surface characteristic is used, it is not necessary to stop ink-jet regurgitation liquid in the position limited as much as possible, and becomes more advantageous to detection of the reaction of the probe and target matter with which making it go and continue fully performs ink-jet regurgitation liquid on a well base behind rather. The hydrophilicity-ized processing at the base of a well indicated to this example is a method desirable as the one embodiment. Moreover, it turns out that the ink-jet process was used for each coloring matter solution, and it has supplied mutually from the well to which each coloring matter is supplied at each well, without having not observed other coloring matter but producing cross contamination.
[0138] Example 11 (the process of the probe array using the solid phase which supplied and obtained the

liquid for the functional-group introduction for probe fixation by the ink-jet method to each well of BM formation substrate, and its use)
(1) The substrate equipped with the black matrix like the example 7 was prepared.
[0139] (2) the silane coupling agent (tradename : KBM603; Shin-Etsu Chemical Co., Ltd. make) containing the silane compound (N-beta-(aminoethyl)-gamma-aminopropyl trimethoxysilane) which combined the amino group — 1wt% — the 10wt% methanol solution to contain was stirred under the room temperature for 3 hours, and the methoxy machine in the above-mentioned silane compound was hydrolyzed Subsequently, the ink tank for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) was filled up with this solution, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the substrate prepared above (1) was also set to the printer, and supplied the silane-coupling-agent solution containing the silane compound with which the methoxy machine was understood an added water part to the well. [as well as an example 8] this substrate — the constant temperature of 25 degrees C and 100% of humidity — after leaving it in a constant humidity chamber for 30 minutes, with pure water, spin dryness was washed and carried out, after that, and the amino group was introduced into the base of each well [100 degrees C] [for 30 minutes]

[0140] (3) next, the last concentration becomes a 5wt%DMSO solution with 5wt(s)% about SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate (Aldrich make) -- as -- dissolving — this solution — the above (2) — the same — carrying out — an ink jet printer — each well — every 100 pls — supplying — subsequently — the constant temperature of 30 degrees C and 100% of humidity — the substrate was left in the constant humidity chamber for 2 hours Next, it is pure, a substrate is washed, spin dryness was carried out, and the maleimide machine was introduced into the base of each well.
[0141] (4) two nucleotides prepared [one nucleotide] the probe (array number : 14) (all — the Nippon Flour Mills Co., Ltd. make and HPLC grade) of a mismatch to the probe (array number : 13) of a mismatch, and the oligomer of the array number 12 to the oligomer (array number : 12) of 18 **** which combined the thiol group with the hydroxyl group of a five prime end through the phosphoric-acid machine and the hexamethylene as a DNA probe, and the oligomer of the array number 12 the following — array number: — the base sequence of 12-14 and the structure of linkage are shown
Array number : 125'HS- 6-O-PO2-O-TGTAAAACGACGGCCAGT3' array number : 135'HS-(CH2)6-O-PO2-O-TGTAAAACCACGGCCAGT (CH2) 3' array number: Each DNA probe of the above-mentioned array numbers 12-14 was dissolved in the phosphate buffer solution of 145'HS-(CH2)6-O-PO2-O-TGTATAACCACGCCCAGT3'(5)10mM so that the last concentration might be set to 10microM. The well of the substrate which created each DNA probe solution above (3) like the above-mentioned example 8 was supplied. When each well was observed under the microscope, although the supplied DNA probe solution rises and exists from opening of a well, it has stopped in the well by the hydrophobic matrix, and cross contamination was not observed. this substrate — the constant temperature of 30 degrees C and 100% of humidity — pure water performed washing and spin dryness to the constant humidity chamber ****** and after that for 2 hours, the thiol group of each DNA probe was made to react with the maleimide machine of each well, and the DNA probe was combined with the substrate
[0142] (6) the DNA probe of the array number 12 — receiving — completeness — TE solution which compounds a complementary single stranded DNA and contains NaCl by the concentration of 50mM(s) — this single stranded DNA — last — a wave — it dissolved so that a degree might be set to 10microM It was immersed, the DNA probe joint substrate obtained above (5) in this solution was lowered over 2 hours to 80 degrees C - 25 degrees C, and hybridization was performed. Next, after washing a substrate for 20 minutes at 20 degrees C using TE solution (pH 8) which contains NaCl by the concentration of 10mM(s), the spin dryer removed the penetrant remover on the front face of a substrate.
[0143] (7) YOYO-1 which is the reagent which emits fluorescence only after intercalates in a hybrid was dissolved so that the last concentration might become TE solution included by concentration 50mM with 10microM about NaCl (pH 8). It supplied 100 pls of this solution at a time to each well of a substrate which processed the above (6) using the ink jet printer like the above (2), and the observation fixed quantity of the fluorescence was carried out like the example 8 (B excitation filter is used). In addition, the signal amplification level of Argus50 is the same as that of an example 8.
[0144] Consequently, from the well which combined the DNA probe of the array number 14, the almost same fluorescence intensity of 1800-2000 as the background was observed. The fluorescence intensity of 3100-3300 was observed from the well which the fluorescence intensity of 7500-8000 is observed [well] from the

well which, on the other hand, combined the DNA probe of the array number 12, and combined the DNA probe of the array number 13. Furthermore, when solid phase is washed for 10 minutes at 35 degrees C using TE buffer solution and fluorescence intensity is measured again, from the well which combined the DNA probe of the array number 13, only fluorescence intensity of the same grade as the background is observed.
[0145] These results showed that a hybridization reaction could be performed in each well and the array number 9 and a perfect complementary target-nucleus acid could be further detected specifically by using the probe array concerning this example.
[0146] The substrate which combined the DNA probe of the array numbers 12-14 like the example 12 (1) example 11 was prepared.
[0147] (2) Three kinds of perfect complementary single stranded DNAs were compounded to the DNA probe of the array numbers 12-14. The three above-mentioned kinds of single stranded DNAs were dissolved in TE solution which contains NaCl by the concentration of 50mM(s) so that each concentration might be set to 10microM. In addition, pH of each single stranded DNA solution is 8. Three ink tanks for bubble-jet printers (tradename : BJC620; Canon [, Inc.], Inc. make) were prepared, each ink tank was filled up with three sorts of above-mentioned single stranded DNA solutions, and the head of the bubble-jet printer used in the example 1 was equipped. Moreover, the substrate prepared above (1) was also set to the printer, and supplied every 100 pls per well of solutions which contain a perfect complementary single stranded DNA respectively to the well which the DNA probe of the array numbers 12-14 has combined. When the state of each well was observed under the microscope at this time, it turns out that the solution of the matter which bleeding of liquid and cross contamination are not observed and should be made to react to each well of a probe array individually can be supplied.
[0148] (3) After making a hybridization reaction perform in each well like an example 11 next, YOYO-1 solution was supplied to each well like the example 11, and the hybrid was detected by observing fluorescence. Consequently, the fluorescence of the intensity of 7500-8000 was observed from all wells. Supplied the reacting matter to each well of a solid phase probe array individually from this, the probe and the reacting matter were made to react in each well, and it was checked that an object is detectable as a result of a reaction.
[0149] Example 13 (process of the probe array using the substrate which was flooded with the solution for epoxy-group introduction in BM formation substrate, and introduced the epoxy group into the well)
(1) The substrate with a black matrix was created according to the publication of an example 7 of (2).
[0150] (2) According to the publication of an example 7 of (1), the 1wt% solution of the silane coupling agent (tradename : KBM403; Shin-Etsu Chemical Co., Ltd. make) containing the silane compound (gamma-glycidoxypropyltrimetoxysilane) which combined the epoxy group was stirred under the room temperature for 1 hour, and the methoxy machine in the molecule of this silane compound was hydrolyzed. Subsequently, the solid phase prepared above (1) into this solution was immersed for 30 minutes under the room temperature, pure water washed this solid phase after that, and water was removed by the nitrogen gas style, and the epoxy group was introduced into the well base. [120 degrees C] [for 5 minutes] At this time, there was a contact angle to the water on the front face of BM in the state of being hard to get wet with 95 degrees, and the contact angle to the water of a well pars basilaris ossis occipitalis was in 33 degrees and the state of being easy to get wet. Thus, introduction of the epoxy group at the base of a well is possible also by processing the solid phase after BM formation by the silane coupling agent.
[0151] (3) The DNA probe of array number 9-11 was combined with the base of a well according to the method indicated to (3) of the above-mentioned example 8, and (4).
[0152] (4) The single stranded DNA which has a complementary base sequence to the array number 9 was compounded by DNA automatic composition Quercus acutissima, and the labeling single stranded DNA which combined the tetramethyl rhodamine with the five prime end through the hexanol amine linker was obtained. This labeling single stranded DNA was dissolved so that the last concentration might be set to 2microM in NaCl at TE solution (pH 8) included by the concentration of 50mM(s). It was immersed, the DNA probe joint substrate obtained above (3) in this solution was lowered over 2 hours from 80 degrees C to 25 degrees C, and the hybridization reaction was performed. They are 10mM(s) about a probe array after that. It washed for 20 minutes at 29 degrees C using the NaCl/TE buffer solution (pH 8), and the probe nucleic acid and the single stranded DNA which was not hybridized were flushed. Next, the fixed quantity of the amount of fluorescence from each well was carried out like the example 8.
[0153] (5) From the well which combined the DNA probe of the array number 9 which are a resulting-

indicator-ized single stranded DNA and a full match, the amount of fluorescence of 8500-9400 was checked. Moreover, from the well which the amount of fluorescence of 2800-3400 is observed [well] from the well which combined the DNA probe of the array number 10, and combined the DNA probe of the array number 11 again, only the about 1200 to 1500 amount of fluorescence was observed. Moreover, when the above-mentioned probe array was washed for 10 minutes at 35 more degrees C using 10 mMNaCl/TE buffer solution (pH 8), the amount of fluorescence from the well which combined the DNA probe of the array number 10 fell even to the level of the background. Therefore, even if it uses the probe array concerning the example of ******, it turns out that specific detection of the hybrid target matter is possible.

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is outline explanatory drawing of the method of manufacturing a probe array using a bubble jet head.
[Drawing 2] It is the A-A line cross section of the bubble jet head of drawing 1 .
[Drawing 3] It is the graph which contrasts the theoretical value of the amount of the nucleic-acid probe which spotted on the aluminum board by the bubble jet process in the example 3, and the actual amount of recoveries.
[Drawing 4] It is the graph which contrasts the theoretical value of the amount of the nucleic-acid probe which spotted on the aluminum board by the bubble jet process in the example 4, and the actual amount of recoveries.
[Drawing 5] (a) one operative condition of the probe array concerning this invention — an outline plan [like] — it is .
(b) It is BB line cross section of drawing 5 (a).
[Drawing 6] It is explanatory drawing of the spotting method in an example 8.
[Description of Notations]
101 Nozzle
103 Solid Phase
104 Drop
105 Bubble Jet Head
107 Liquid Containing Nucleic-Acid Probe Breathed Out
109 Protective Coat
111-1,111-2 Electrode
113 Exoergic Resistor Layer
115 Accumulation Layer
116 Substrate Currently Formed with Good Alumina of Thermolysis Nature Etc.
117 Exoergic Head
119 Regurgitation Orifice
121 Meniscus
123 Foaming Field

[Translation done.]

* NOTICES *

CORRECTION or AMENDMENT

[Official Gazette Type] Printing of amendment by the convention of 2 of Article 17 of patent law.
[Section partition] The 1st partition of the 1st section.
[Date of issue] April 10, Heisei 13 (2001. 4.10)

[Publication No.] JP,11-187900,A.
[Date of Publication] July 13, Heisei 11 (1999. 7.13)
[**** format] Open patent official report 11-1879.
[Filing Number] Japanese Patent Application No. 10-209923.
[The 7th edition of International Patent Classification]

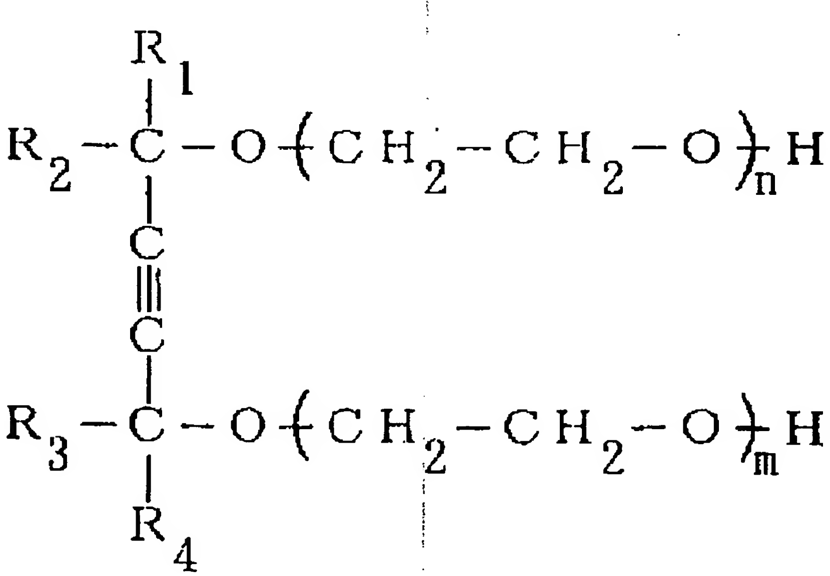
C12Q 1/68
G01N 33/50
33/543 525
33/566
//C12N 15/09

[FI]

C12Q 1/68 A
G01N 33/50 P
33/543 525 G
33/566
C12N 15/00 A

[Procedure revision]
[Filing Date] July 31, Heisei 12 (2000. 7.31)
[Procedure amendment 1]
[Document to be Amended] Specification.
[Item(s) to be Amended] The name of invention.
[Method of Amendment] Change.
[Proposed Amendment]
[Title of the Invention] Equipment for manufacturing the spotting method to the solid phase of a probe, a probe array, its manufacture method and the method of detection of the target matter using it, the specification-ized method of the structure of the target matter, and a probe array.
[Procedure amendment 2]
[Document to be Amended] Specification.
[Item(s) to be Amended] Claim.
[Method of Amendment] Change.
[Proposed Amendment]
[Claim(s)]
[Claim 1] It is the spotting method to the solid phase of the probe which has the process which the liquid which contains a combinable probe specifically to the target matter is supplied [process] to a solid phase front face by the ink-jet method, and makes it adhere to this solid phase front face.
This solid phase front face and this probe are the spotting method to the solid phase of the probe which has

a functional group respectively and is characterized by these functional groups being what reacts mutually.
[Claim 2] The spotting method according to claim 1 that this probe is a single strand nucleic-acid probe.
[Claim 3] The spotting method according to claim 2 that this single strand nucleic-acid probe contains a single stranded DNA probe or an RNA probe.
[Claim 4] The spotting method according to claim 2 that this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 5] The spotting method according to claim 2 to 4 that the functional group which this solid phase front face has is a maleimide machine, and the functional group which this single strand nucleic-acid probe has is a thiol (SH) machine.
[Claim 6] It is the spotting method according to claim 5 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 7] It is the spotting method according to claim 5 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 8] The spotting method according to claim 5 to which the maleimide machine on this glass substrate and the thiol group of this single strand nucleic-acid probe are made to react at least for 30 minutes.
[Claim 9] The spotting method according to claim 8 that this single strand nucleic-acid probe makes this maleimide machine and this thiol group react to an end for at least 2 hours or more including the single strand PNA probe which has a thiol group.
[Claim 10] The spotting method according to claim 9 that the thiol group of this single strand PNA probe end is what is introduced by combination of the cysteine by the side of the amino terminus of a single strand PNA probe.
[Claim 11] The spotting method according to claim 2 to 4 that the functional group which this solid phase front face has is an epoxy group, and the functional group which this single strand nucleic-acid probe has is an amino group.
[Claim 12] It is the spotting method according to claim 11 which this solid phase is a glass plate, and this epoxy group applies the silane compound which has an epoxy group in a molecule on the front face of this glass plate, and this compound and this glass plate are made to react, and is introduced.
[Claim 13] This epoxy group is the spotting method according to claim 11 introduced by the application of a up to [this solid phase of the polyglycidylmethacrylate which has an epoxy group].
[Claim 14] this liquid — this liquid — receiving — a urea — five to 10 weight (wt) %, and a glycerol — 5 - 10wt% and a thiodiglycol — 5 - 10wt% and acetylene alcohol — 1wt% — the included spotting method according to claim 1 to 13
[Claim 15] The spotting method according to claim 14 which is what has the structure by which this acetylene alcohol is shown by the following general formula (I).
[Formula 1]



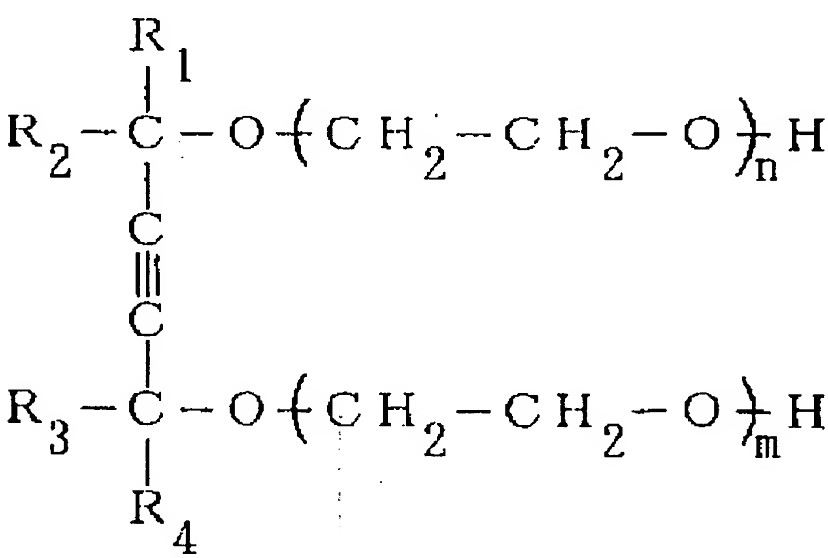
(I)

(R1, R2, R3, and R4 express an alkyl group among the above-mentioned formula, and m and n express an integer, respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)
[Claim 16] The spotting method according to claim 2 to 15 that the concentration of this single strand nucleic-acid probe in this liquid is 0.05-500microM.
[Claim 17] The spotting method according to claim 16 that the concentration of this single strand nucleic-acid probe in this liquid is 2-50microM.
[Claim 18] The spotting method according to claim 2 to 17 that the length of this single strand nucleic-acid probe is 2 - 5000 base length.
[Claim 19] The spotting method according to claim 18 that the length of this single strand nucleic-acid probe is 2 - 60 base length.
[Claim 20] The spotting method according to claim 1 to 19 that this ink-jet method is a bubble jet process.
[Claim 21] The spotting method according to claim 1 that this probe is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 22] The spotting method according to claim 1 that this probe is protein.
[Claim 23] The spotting method according to claim 22 that this protein is an antibody or an enzyme.
[Claim 24] The spotting method according to claim 1 that this probe is an antigen.
[Claim 25] The spotting method according to claim 21 to 24 that the functional group which this solid phase front face has is a maleimide machine, and the functional group which this probe has is a thiol (SH) machine.
[Claim 26] It is the spotting method according to claim 25 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 27] It is the spotting method according to claim 25 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 28] The spotting method according to claim 1 to 27 of spotting this liquid by the density of 10000 or more per 1 square inch on this solid phase so that it may become the spot which became independent mutually.
[Claim 29] This solid phase is the spotting method according to claim 1 to 28 of having the surface characteristic with an evenly uniform front face.
[Claim 30] The spotting method according to claim 1 that this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and supplies this liquid to each well.
[Claim 31] The probe array characterized by having the spot of the probe which has been independent mutually to two or more parts on the front face of solid phase by the density of 10000 or more pieces in a 1 square inch.
[Claim 32] This solid phase is a probe array according to claim 31 which has a flat front face and has the uniform surface characteristic.
[Claim 33] The probe array according to claim 31 or 32 this probe of whose is a single strand nucleic-acid probe.
[Claim 34] The probe array according to claim 33 in which this single strand nucleic-acid probe contains a single stranded DNA probe or a single-stranded-RNA probe.
[Claim 35] The probe array according to claim 33 in which this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 36] The probe array according to claim 33 to 35 which this single strand nucleic-acid probe has combined with this solid phase front face according to covalent bond by the reaction of the functional groups which each of this solid phase front face and a single strand nucleic-acid probe has.
[Claim 37] The probe array according to claim 36 whose functional group which this single strand nucleic-acid probe has the functional group which this solid phase front face has is a maleimide machine, and is a thiol (SH) machine.
[Claim 38] The probe array according to claim 37 to which this single strand PNA probe has [this single strand nucleic-acid probe] a cysteine residue in the amino terminus side including a single strand PNA probe.

[Claim 39] The probe array according to claim 36 whose functional group which this probe has the functional

group which this solid phase front face has is an epoxy group, and is an amino group.
[Claim 40] The probe array according to claim 31 or 32 this probe of whose is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 41] The probe array according to claim 31 or 32 this probe of whose is protein.
[Claim 42] The probe array according to claim 41 this protein of whose is an antibody or an enzyme.
[Claim 43] The probe array according to claim 31 or 32 this probe of whose is an antigen.
[Claim 44] The probe array according to claim 41 to 43 which this probe has combined with this solid phase front face according to covalent bond by the reaction of the functional groups which each has [this solid phase front face and this probe].
[Claim 45] The probe array according to claim 44 whose functional group which this probe has the functional group which this solid phase front face has is a maleimide machine, and is a thiol (SH) machine.
[Claim 46] It is the probe array according to claim 37 or 45 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 47] It is the probe array according to claim 37 or 45 which this solid phase is a glass plate, and the this amino group after a **** maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 48] The claims 36-39 by which blocking is given to this functional group in front faces other than the part in which this spot of this solid phase exists, and pro-BUAREI given in either 44-47.
[Claim 49] The probe array according to claim 31 to 48 formed of grant of a up to [this solid phase of the liquid with which this spot contains this probe].
[Claim 50] The probe array according to claim 49 by which grant of a up to [this solid phase of this liquid] is made by the ink-jet method.
[Claim 51] The probe array according to claim 50 this ink-jet method of whose is a bubble jet process.
[Claim 52] The probe array according to claim 31 each spot of whose this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and corresponds with the position of each well.
[Claim 53] It is the manufacture method of the probe array which has the spot which contains a combinable probe independently specifically to the target matter in two or more parts on the front face of solid phase.
It has the process which makes the liquid containing this probe supply and adhere to the position on this front face of solid phase using the ink-jet method.
This solid phase front face and this probe have a functional group respectively, and these functional groups react mutually.
The manufacture method of the probe array characterized by things.
[Claim 54] The manufacture method according to claim 53 that this probe is a single strand nucleic-acid probe.
[Claim 55] The manufacture method according to claim 54 that this single strand nucleic-acid probe contains a single stranded DNA probe or an RNA probe.
[Claim 56] The manufacture method according to claim 54 that this single strand nucleic-acid probe contains a single strand PNA probe.
[Claim 57] It is the manufacture method according to claim 54 which is that to which this solid phase front face and this single strand nucleic-acid probe have a functional group respectively, and these functional groups react by contact.
[Claim 58] The manufacture method according to claim 57 that the functional group which this solid phase front face has is a maleimide machine, and the functional group which this single strand nucleic-acid probe has is a thiol (SH) machine.
[Claim 59] It is the manufacture method according to claim 58 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 60] It is the manufacture method according to claim 58 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 61] The manufacture method according to claim 58 to 60 to which the maleimide machine on this glass

substrate and the thiol group of this single strand nucleic acid are made to react at least for 30 minutes.
[Claim 62] The manufacture method according to claim 58 to 60 that this single strand nucleic acid makes this maleimide machine and this thiol group react to an end for at least 2 hours or more including the single strand PNA probe which has a thiol group.
[Claim 63] The manufacture method according to claim 62 that the thiol group of this single strand PNA probe end is what is introduced by combination of the cysteine by the side of the amino terminus of a single strand PNA probe.
[Claim 64] The manufacture method according to claim 57 that the functional group which this solid phase front face has is an epoxy group, and the functional group which this single strand nucleic-acid probe has is an amino group.
[Claim 65] It is the manufacture method according to claim 64 which this solid phase is a glass plate, and this epoxy group applies the silane compound which has an epoxy group in a molecule on the front face of this glass plate, and this compound and this glass plate are made to react, and is introduced.
[Claim 66] This epoxy group is the manufacture method according to claim 64 introduced by the application of a up to [this solid phase of the polyglycidylmethacrylate resin which has an epoxy group].
[Claim 67] this liquid -- this liquid -- receiving -- a urea -- five to 10 weight (wt) %, and a glycerol -- 5 - 10wt% and a thiodiglycol -- 5 - 10wt% and acetylene alcohol -- 1wt% -- the included manufacture method according to claim 53 to 66
[Claim 68] The manufacture method according to claim 67 which is what has the structure by which this acetylene alcohol is shown by the following general formula (I).
[Formula 2]



(I)

(R1, R2, R3, and R4 express an alkyl group among the above-mentioned formula, and m and n express an integer, respectively, and it is m= 0 and n= 0, or 1 <=m+n<=30, and, in the case of m+n=1, is m or n0.)
[Claim 69] The manufacture method according to claim 54 to 56 that the concentration of this single strand nucleic-acid probe in this liquid is 0.05-500microM.
[Claim 70] The manufacture method according to claim 69 that the concentration of this single strand nucleic-acid probe in this liquid is 2-50microM.
[Claim 71] The manufacture method according to claim 54 to 56 that the length of this single strand nucleic-acid probe is 2 - 5000 base length.
[Claim 72] The manufacture method according to claim 71 that the length of this single strand nucleic-acid probe is 2 - 60 base length.
[Claim 73] The manufacture method according to claim 53 to 72 that this ink-jet method is a bubble jet process.
[Claim 74] The manufacture method according to claim 53 to 73 of spotting the liquid containing this single strand nucleic-acid probe by the density of 10000 or more per 1 square inch on this solid phase so that it may become the spot which became independent mutually.

[Claim 75] The manufacture method according to claim 53 that this probe is the oligopeptide or polypeptide which has a specific amino acid sequence.
[Claim 76] The manufacture method according to claim 53 that this probe is protein.
[Claim 77] The manufacture method according to claim 76 that this protein is an antibody or an enzyme.
[Claim 78] The manufacture method according to claim 53 that this probe is an antigen.
[Claim 79] The manufacture method according to claim 75 to 78 that the functional group which this solid phase front face has is a maleimide machine, and the functional group which this probe has is a thiol (SH) machine.
[Claim 80] It is the manufacture method according to claim 79 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces them.
[Claim 81] It is the manufacture method according to claim 79 which this solid phase is a glass plate, and the this amino group after this maleimide machine introduces the amino group into the front face of this glass plate, and SUKUSHIIMIJIRU-4-(maleimide phenyl) butyrate are made to react, and introduces them.
[Claim 82] This solid phase is the manufacture method according to claim 53 to 81 of having the surface characteristic with an evenly uniform front face.
[Claim 83] The manufacture method according to claim 79 which blocks so that a nucleic acid may not adhere to any parts other than the part by which this single strand nucleic acid is fixed, after making this single strand nucleic acid fix to solid phase.
[Claim 84] The manufacture method according to claim 83 of having the process to which this blocking dips the solid phase to which this single strand nucleic acid was fixed in bovine-serum-albumin solution.
[Claim 85] The manufacture method according to claim 84 that the concentration of this bovine serum albumin is 0.1 – 5%.
[Claim 86] The manufacture method according to claim 84 of performing being immersed [solution / bovine-serum-albumin / of this solid phase] for at least 2 hours.
[Claim 87] The manufacture method according to claim 53 to 81 that this solid phase is divided by the matrix arranged in the shape of a pattern on the front face, is equipped with two or more wells which use as a base this solid phase front face that it comes to expose in the shape of a pattern, and supplies this liquid to each well.
[Claim 88] Each spot and this sample of the probe array which has the probe specifically combined to the target matter which may be contained in the sample as two or more spots which became independent mutually on solid phase are contacted. The method of detection of the target matter characterized by being the method of detecting a reactant with this target matter and this probe, and detecting the existence of this target matter in this sample on this solid phase, and being a probe array with this probe array given in either of the aforementioned claims 31-52.
[Claim 89] The process which prepares the probe array which is the method of specifying the structure of the target matter contained in a sample, and was equipped with the spot of the probe specifically combined with a solid phase front face to this target matter; The process which detects combination with process; which contacts this sample at this spot and this target matter, and this probe, The specification-ized method of the structure of the target matter where it is the specification-ized method of the structure of the target matter of ****(ing), and this probe array is characterized by being the probe array of a publication at either of the aforementioned claims 31-52.
[Claim 90] It is the manufacture method of a probe array that two or more spots of each other containing the probe are arranged in the solid phase front face independently.
(i) — process; which prepares the solid phase which has the maleimide machine of N-(maleimide KAPURO yloxy) SUKUSHI imide origin on a front face — and .
(ii) The manufacture method of the probe array characterized by having the process which gives the liquid containing the probe which has a thiol group to the position of the front face of the solid phase acquired according to this process (i), and forms this spot in a solid phase front face.
[Claim 91] It is the method of combining a probe with the specific position on the front face of solid phase.
(i) Process; which prepares the probe which has a thiol group.
(ii) — process; which introduces the amino group into a solid phase front face — and .
(iii) process; which the amino group and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces a maleimide machine into a solid phase front face — and .

(iv) The joint method to the specific position on the front face of solid phase of the probe characterized by having the process which supplies the liquid of the predetermined amount containing this probe to a position predetermined [of the solid phase acquired according to the process (iii)].
[Claim 92] It is the method of forming a probe spot in the specific position on the front face of solid phase.
(i) Process; which prepares the probe which has a thiol group.
(ii) Process; which introduces the amino group into a solid phase front face.
(iii) Process; which this amino group and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces a maleimide machine into a solid phase front face.
(iv) The formation method of the probe spot on the front face of solid phase characterized by having the process which supplies the liquid of the specified quantity containing this probe to the position of the solid phase acquired according to the process (iii).
[Claim 93] It is the manufacture method of a probe array that two or more spots in which each contains the combinable probe specifically to the target matter are arranged independently on the solid phase front face.
(i) Process; which prepares the probe which has a thiol group.
(ii) — process; which prepares the solid phase which possesses the maleimide machine on the whole front face — and .
(iii) The process which gives the liquid containing this probe to the front face of this solid phase by the ink-jet method,
The manufacture method of the probe array characterized by ****(ing).
[Claim 94] It is the method of spotting the probe which can be specifically combined to a target to solid phase.
(i) — process; which supplies the liquid containing this probe to a solid phase front face by the bubble jet process — and .
(ii) How to spot the probe characterized by having the process which forms the spot of this probe in this solid phase front face to solid phase.
[Claim 95] It is the method of manufacturing the probe array by which two or more spots which contain a combinable probe specifically to the target matter are arranged independently in each position on the front face of solid phase.
(i) — process; which supplies the liquid containing this probe to a solid phase front face by the bubble jet process — and .
(ii) Process which forms the spot of this probe in this solid phase front face,
How to manufacture the probe array characterized by ****(ing).
[Claim 96] Each spot and this sample of the probe array which has the probe specifically combined to the target matter which may be contained in the sample as two or more spots which became independent mutually on solid phase are contacted. It is the method of detecting a reactant with this target matter and this probe, and detecting the existence of this target matter in this sample on this solid phase. as this probe array The method of detection of the target matter characterized by using the probe array manufactured by the method of a publication by either of the aforementioned claims 53-87.
[Claim 97] It is the method of specifying the structure of the target matter contained in a sample.
Process; which prepares the probe array equipped with the spot of the probe specifically combined with a solid phase front face to this target matter.
process; which contacts this sample at this spot — and .
It has the process which detects combination with this target matter and this probe.
The specification-ized method of the structure of the target matter characterized by using the probe array manufactured by the method of a publication as this probe array by either of the aforementioned claims 53-87.
[Claim 98] It is equipment for spotting a combinable probe specifically to the target matter on a solid phase front face.
Applied-part [of solid phase];.
tank; which has held the liquid containing this probe — and .
The head on which this liquid can be breathed out by the bubble jet process, and is closed towards the front face of this solid phase,
Equipment characterized by ******(ing).
[Claim 99] It is equipment for manufacturing the probe array which equips two or more parts on the front face

of solid phase with the spot of the probe which has been independent mutually.
Conveyance means; [of solid phase];.
tank; which has held the liquid containing this probe -- and .
The head on which this liquid can be breathed out by the bubble jet process, and is closed towards the front face of this solid phase,
Equipment characterized by ******(ing).
[Procedure amendment 3]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0014.
[Method of Amendment] Change.
[Proposed Amendment]
[0014]
[Means for Solving the Problem] It is the spotting method to the solid phase of the probe which has the process which supplies the liquid with which the spotting method which takes like 1 operative condition as for this invention that the above-mentioned purpose can be attained contains a combinable probe specifically to the target matter to a solid-phase front face by the ink-jet method, and is made to adhere to this solid-phase front face, and this solid-phase front face and this probe have a functional group respectively, and are characterized by for these functional groups to be what reacts mutually.
[Procedure amendment 4]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0017.
[Method of Amendment] Change.
[Proposed Amendment]
[0017] Moreover, the manufacture method of the probe array which takes like 1 operative condition as for this invention It is the manufacture method of the probe array which has the spot which contains a combinable probe independently specifically to the target matter in two or more parts on the front face of solid phase. It has the process which makes the liquid containing this probe supply and adhere to the position on this front face of solid phase using the ink-jet method, this solid phase front face and this probe have a functional group respectively, and these functional groups are characterized by being what reacts mutually. The probe array by which the spot has been arranged with high density can be manufactured efficiently, without harming a probe according to this mode.
[Procedure amendment 5]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0018.
[Method of Amendment] Change.
[Proposed Amendment]
[0018] Moreover, the method of detection of the target matter which takes like 1 operative condition as for this invention which can attain the above-mentioned purpose For example, each spot and this sample of the probe array which has the probe specifically combined to the target matter which may be contained in the sample as two or more spots which became independent mutually on solid phase are contacted. It is the method of detecting a reactant with this target matter and this probe, and detecting the existence of this target matter in this sample on this solid phase, and is characterized by this probe array being a probe array of the above-mentioned composition. moreover, other operative conditions of this invention -- the method of detection of the target matter applied like -- for example, it is characterized by using the probe array manufactured by the manufacture method of the above-mentioned probe array According to the mode of ********, the target matter is efficiently detectable.
[Procedure amendment 6]
[Document to be Amended] Specification.
[Item(s) to be Amended] 0019.
[Method of Amendment] Change.
[Proposed Amendment]
[0019] Furthermore, the specification-ized method of the structure of the target matter which takes like 1 operative condition as for this invention that the above-mentioned purpose can be attained For example, it is the method of specifying the structure of the target matter contained in a sample. The process which

prepares the probe array equipped with the spot of the probe specifically combined with a solid phase front face to this target matter; The process which detects combination with process; which contacts this sample at this spot and this target matter, and this probe, It is the specification-ized method of the structure of the target matter of ****(ing), and is characterized by this probe array being a probe array of the aforementioned composition. moreover, other operative conditions of this invention — the specification-ized method of the structure of the target matter applied like is characterized by using the probe array manufactured by the manufacture method of for example, the aforementioned probe array When it is a single strand nucleic acid even from a small amount of sample by using these modes, the structure of the target matter, for example, the target matter, in this sample, the base sequence can be specified efficiently. Moreover, the joint method to the specific position on the front face of solid phase of the probe which takes like 1 operative condition as for this invention is a method of combining a probe with the specific position on the front face of solid phase.
(i) Process; which prepares the probe which has a thiol group.
(ii) — process; which introduces the amino group into a solid phase front face — and .
(iii) process; which the amino group and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces a maleimide machine into a solid phase front face — and .
(iv) It is characterized by having the process which supplies the liquid of the predetermined amount containing this probe to a position predetermined [of the solid phase acquired according to the process (iii)].
Moreover, the formation method of the probe spot on the front face of solid phase which takes like 1 operative condition as for this invention is the method of forming a probe spot in the specific position on the front face of solid phase.
(i) Process; which prepares the probe which has a thiol group.
(ii) Process; which introduces the amino group into a solid phase front face.
(iii) Process; which this amino group and N-(6-maleimide KAPURO yloxy) SUKUSHI imide are made to react, and introduces a maleimide machine into a solid phase front face.
(iv) It is characterized by having the process which supplies the liquid of the specified quantity containing this probe to the position of the solid phase acquired according to the process (iii). Moreover, other modes of the manufacture method of the probe array of this invention are the manufacture methods of a probe array that two or more spots in which each contains the combinable probe specifically to the target matter are arranged independently on the solid phase front face.
(i) Process; which prepares the probe which has a thiol group.
(ii) — process; which prepares the solid phase which possesses the maleimide machine on the whole front face — and .
(iii) It is characterized by having the process which gives the liquid containing this probe to the front face of this solid phase by the ink-jet method. Moreover, other modes of the method of spotting the probe concerning this invention to solid phase are the methods of spotting the probe which can be specifically combined to a target to solid phase.
(i) It is characterized by being characterized by having the process which forms the spot of this probe in process; and (ii) this solid phase front face which supply the liquid containing this probe to a solid phase front face by the bubble jet process. Moreover, other modes of the manufacture method of the probe array concerning this invention are the methods of manufacturing the probe array by which two or more spots which contain a combinable probe specifically to the target matter are arranged independently in each position on the front face of solid phase.
(i) — process; which supplies the liquid containing this probe to a solid phase front face by the bubble jet process — and .
(ii) It is characterized by having the </U> process which forms the spot of this probe in this solid phase front face.
Moreover, other modes of the method of detection of the target matter concerning this invention For example, each spot and this sample of the probe array which has the probe specifically combined to the target matter which may be contained in the sample as two or more spots which became independent mutually on solid phase are contacted. It is the method of detecting a reactant with this target matter and this probe, and detecting the existence of this target matter in this sample on this solid phase, and is characterized by using the probe array manufactured by the aforementioned method as this probe array.
Moreover, other modes of the specification-ized method of the structure of the target matter concerning this invention For example, it is the method of specifying the structure of the target matter contained in a sample.

The process which prepares the probe array equipped with the spot of the probe specifically combined with a solid phase front face to this target matter; The process which detects combination with process; which contacts this sample at this spot and this target matter, and this probe, It **** and is characterized by using the probe array manufactured by the aforementioned method as this probe array. Moreover, the equipment for spotting the spot of a probe on a solid-phase front face which takes like 1 operative condition as for this invention is characterized by to provide the head which can breathe out this liquid on a solid-phase front face by the bubble jet process, and closes it on it towards the front face of tank; which is equipment for spotting a combinable probe specifically to the target matter, and has held the liquid containing the applied-part; this probe of solid phase, and this solid phase. Moreover, the equipment for manufacturing a probe array which takes like 1 operative condition as for this invention is equipment for manufacturing the probe array which equips two or more parts on the front face of solid phase with the spot of the probe which has been independent mutually, and is characterized by to provide the head on which this liquid can be breathed out by the bubble jet process, and is closed towards the front face of tank; which has held the liquid containing the conveyance means; this probe of solid phase, and this solid phase.

[Translation done.]